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Environmentally Shrewdly Innoxious Phyto-Chemical Route Synthesis of Silver Nanoparticles and Their Ingress on Multi-Drug Resistance Bacteria

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Abstract
Nanoparticles synthesis by means of plant extracts is one of the greatest unpretentious, convenient, environmentally friendly, and economically beneficial approaches that alleviate the engrossment of noxious chemicals. Hence, in recent years, several eco-friendly processes for the rapid synthesis of silver nanoparticles have been reported using aqueous extracts of plant parts such as the leaf, bark, roots, etc. This review summarizes and elaborates the new findings in this research domain of the green synthesis of silver nanoparticles (AgNPs) using different plant extracts and their potential applications as antimicrobial agents. While highlighting the recently used different plants for the synthesis of highly efficient antimicrobial green AgNPs, we aim to provide a systematic in-depth discussion on the possible influence of the phytochemicals and their concentrations in the plants extracts, extraction solvent, and extraction temperature, as well as reaction temperature, pH, reaction time, and concentration of precursor on the size, shape and stability of the produced AgNPs. Exhaustive details of the plausible mechanism of the interaction of AgNPs with the cell wall of microbes, leading to cell death, and high antimicrobial activities have also been elaborated. The leaves of Ionidium Suffruticosum were collected, shadow dried, crushed and further nano size powdered by mixer mill and then liquidity extracted through maceration process. The prepared leaf extract used to synthesis the silver nanoparticles(AgNPs) and characterized by using UV, XRD, SEM, and DLS as well as subjected to evaluate the presence of phytochemicals and antibacterial activity against selected multi drug resistance bacterial strains.
KEY WORDS: *antibacterial, extract, mixer mill, nanoparticles, phytochemicals*

1.INTRODUCTION

Plants produce an immensely ridiculous assortment of specific secondary metabolites encompassing an massive number of energetic and complementary Phyto compounds. Humans hold distinct pharmacological acquaintance of the healing properties of plants. The utilization of plant extracts, based ethnomedicine comprising hundreds of biochemicals as medicinal agents[1-3]. The conversion of traditional ethno-pharmaceuticals into nano medicine and its invention has shadowed a modern technical pathway, abetted through nano techniques for the isolation and characterization of medicinal particles [4].Some nanotechnology researchers recognized by means of incessant nanoparticles exploration, through the energetic phyto compounds arising subsequent extensive application of nanotechnology based analysis and testing [5-9]. In current years, actionable metal based nanoparticles were synthesized and determined using various nano techniques such as high energy ball-milling, XRD,TEM,SEM, Zeta potential, DLS etc.,[10-14]. Also, tracked by the proof of identity that the plants comprising the active components, recognized as reputed reduction and nucleation agents during nanoparticle synthesis[15]. Nanotechnology has sustained to harvest extensive consideration completed its earlier limited periods owing through its widespread as well as beneficial claims in plant systems [16]. Nano techniques offers some dais in advance of effort at atomic and super molecular range referring to plant nanomaterial, in the range between 1 to100 nanometre [17]. This prospect facing brand new progressive plant based nanoparticles products with improved functions, physical and biological

characteristics[18]. Nanotechnology have formed the motivation enroute for the incessant attention to plant science research area , specifically in relation to the nanoparticle synthesis[19]. Therefore, plant biomaterial products give the impression and could be an imminent nanomedicine. Intrinsically, one of the distinguished instances of nano biomaterials acknowledged significant consideration into current years and have to be extensively used is plant created nanocomposites and nanomedicines [20]. In our work, we have discussed the nanotechnical ideas and methods, from novel drug discovery approach using Ionidium Suffruticosum, leaf extract to products silver nanoparticles.

Figure 1. Photograph of Ionidium Suffruticosum leaves

2.MATERIALS AND METHODS

2.1. Collection of leaves

The renewed Ionidium Suffruticosum leaves were placid around Gundur village arid area near Tiruchirappalli, Tamilnadu ,India. The plant were identified by Dr.N.Vidhya Rani, a Private siddha medicine expert.

2.2. Preparation of Leaf Extract

The aqueous leaf extracts were prepared by 10g Ionidium Suffruticosum leaf powder weighed and added to 100ml of double distilled water in a 250ML Erlenmeyer flask. The combination was heated for40 minutes at 60°C in a water bath to circumvent dilapidation of the phytochemicals while stirring sporadically The leaf extract was finally chilled and filtered using Whatman Grade 1 filter paper.

2.3. Synthesis of Silver nanoparticles (AgNPs)

The aqueous 0.05 M silver nitrate ($AgNO_3$) solution was ready by using distilled water for silver nanoparticles synthesis. The Ionidium Suffruticosum leaves extracts was mixed to freshly prepared 0.05 M AgNO3 solution in a conical flask, the combination heated uninterruptedly at 70 °C for 20 min. The mixture solution in conical flask changes from yellowish brown to brown. The color changes in the mixture, signifying the development of silver nanoparticles. The synthesized nanoparticles centrifuged at 10,000 rpm for 15 min then the nanoparticles were collected subsequently removing the supernatant. The pellet form of collected nanoparticles were dried and stored in dark glass bottles gelling of nanoparticles. For nano characterization, the collected silver nanoparticles were allowable to dry in a watch glass.

2.4. Nano- Characterization

The synthesized silver nanoparticles were encompassed with various characterization techniques including UV-spectroscopic analysis, X-ray diffraction (XRD), SEM and DLS. It divulges and recognizes chemical conformations, crystal edifices, size, morphology and photoelectric features of nanoparticles.

(a) Morphology of Nanoparticles

The synthesized silver nanoparticles(AgNPs), morphology was detected by using SEM (Scanning Electron Microscope(Hitachi S-4000).The particle nano structure was resolute by electron dispersion at the particle surface .

(b) Particle size Determination

The equipped powder extract of AgNPs size were determine by using EQUINOX 100 X-ray Diffractometer(Thermo Scientific). Each obtained peak position were interpreted with Scherrer Equation:

$$\mathbf{Dp = (0.94\ X\ \lambda) / (\beta\ X\ Cos\theta)}$$

Whereas, Dp (Average Crystallite size), β (Line broadening in radians), θ (Bragg angle), λ (X-Ray wavelength) and further the equation used in the determination of size of crystallites (grains)s in the form of powder.

(c) Spectroscopic analysis

This analysis was carried out by using a UV-Vis spectrometer (Lambda Model: 950,Perkin Elmer, USA) with a range girth of 2 nm, with a 10mm cuvette cell at 37°.The silver nanoparticles examined underneath the UV visible light source at the wavelength vacillating around 300 to 750 nm to confirm the formation of nanoparticles. The nanoparticles was mixed with pure distilled water in 1:3 ratio and centrifugated at 6000 rpm enroute for 30 minutes and riddled over Whatman No. 1 filter paper. Some sample filtrates were used for UV-VIS Spectroscopic analysis.

(d) Dynamic light scattering analysis

The nano sized particle dispersion in liquid form and polydispersity index leaf material were evaluated by DLS method (Malvern Nano system).Some limitations working for the analysis comprised a wavelength of 633 nm, RI(refractive index) liquid medium is 1.1.32, temperature 22 °C, viscosity of liquid medium 0.87873 and light scattering angle 173.

2.5. Phytochemical analyssis

For qualitative phytochemical guesstimate of Ionidium Suffruticosum leaf powder extracts were executed using typical procedures**,** for the finding of medicinally important flavonoids, tannins, amino acids, steroids, alkaloids, terpenoids, saponin, and phenols.

2.6. Antimicrobial activity

The butchery activity of nanotech assisted ethnomedicine was tested against some human bacterial pathogens together with Salmonalla Typhi, Staphylococcus aureus, Shigella and Klebsiella Pneumonia, along with the standard allopathic antibiotics were assessed using Kirby-Bauer well diffusion assay. For the facilitation of diffusion by prepared ethnomedicine in inoculated medium of the plates with 6mm diameter wells were encumbered with 1mg/ml ethnomedicine sideways with positive and negative controls by means of a micro pipette. The positive rheostat delimited 1mg/ml tetracycline while the negative control pure distilled water. All the plates were categorized and allowed nurturing for 24 hrs at 37 °C. At the end of incubation, the zones of inhibitions were compared to matching the butchery activity against tested bacterial pathogens were measured in mm.
observed.

2.7. Statistical analysis

The research outcome data attained from our work has been articulated by mean (± SD error). Data comprises graphs, plotting all are confirmed by means of Version 5.0, Graph Pad Prism Software (San Diego, CA, USA).

3.Results

The mixer milled leaf powder particles were categorized to fix their morphology in addition to size through Scanning Electron Microscope (SEM). Each resultant SEM images shape were gotten which are shown in Fig. 3 and the shape of the leaf particles from place to place look like a spherical or spheroid shape. Likewise, the predictable typical particles size were roughly 21 to 32 nm.

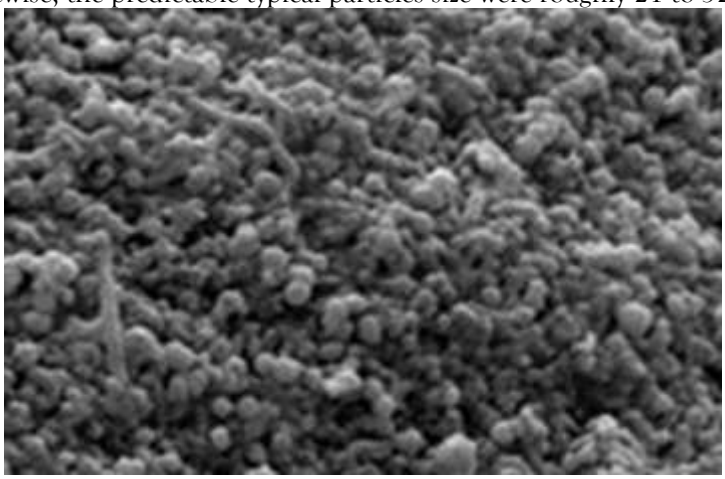


Fig 3. SEM image display of AgNPs

The crystallite of the silver particles size using Ionidium Suffruticosum leaf extract was inspected over X rays diffractions (Fig. 4). Determined nanoparticles size was premeditated by Scherrer equation: ($D = k\lambda/\beta\cos\theta$). The angle of diffraction regarding the (111), 200, 220, and 311 lattice planes respectively. The approximate size of the crystallites (grains) were flinch about 30 nanometre range. The lattice limitations for the leaf powder nanoparticles were stubborn to be c = 0.4086 nm.

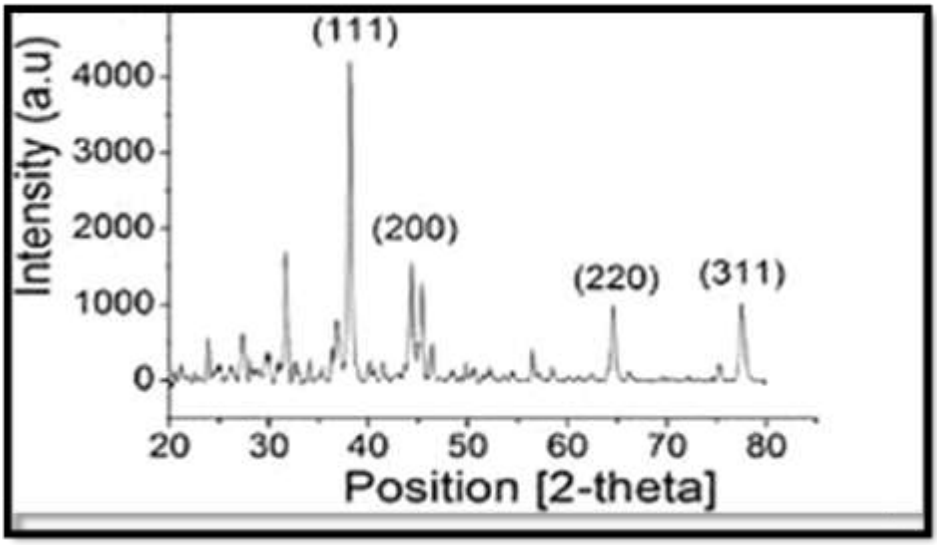


Fig 4.XRD pattern of silver nanoparticles display peaks such as 111, 200, 220, and 311are confirmative to crystallite points

UV–Vis spectrophotometric study was approved for the prime examination of silver nanooparticles . The absorbance of solution was investigated for one week. The absorption spectrum of the nano meter at room temperature showed in Fig. 5, and the spectral range was established from 200 nm to 800 nm. The determined peak wavelength was detected at 376 nm, it confirms that the solution form of silver nano particles consists of polydisperse particles.

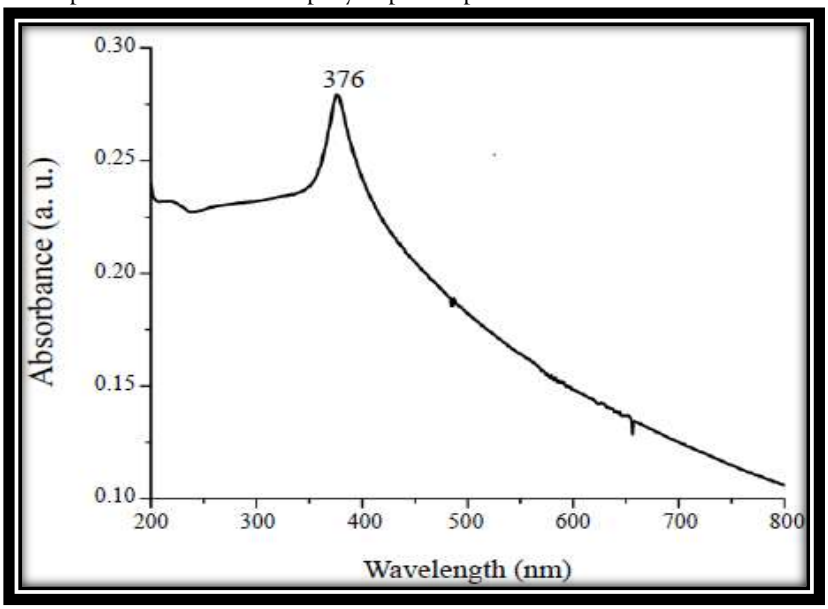


Fig 5. UV-Vis absorption spectra silver nanoparticles

By using DLS- Dynamic light scattering technique the size of dispersal silhouette of nano particles present in any solution or suspension can be determined. By the way of our study. The mean nanoparticles size dispersal in suspension, exposed that size referring liquid form nanoparticles assumed the size range around 49 to 52 nm. The fig. 6, clearly shows, that the suspension had diverse sizes of nanoparticles.

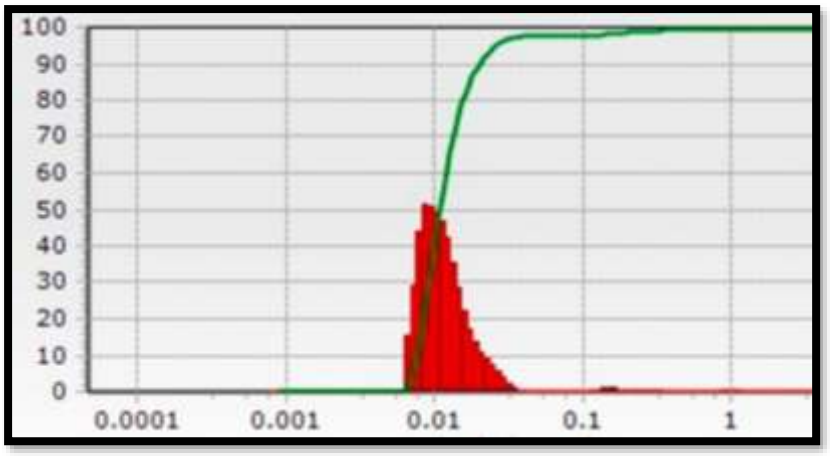
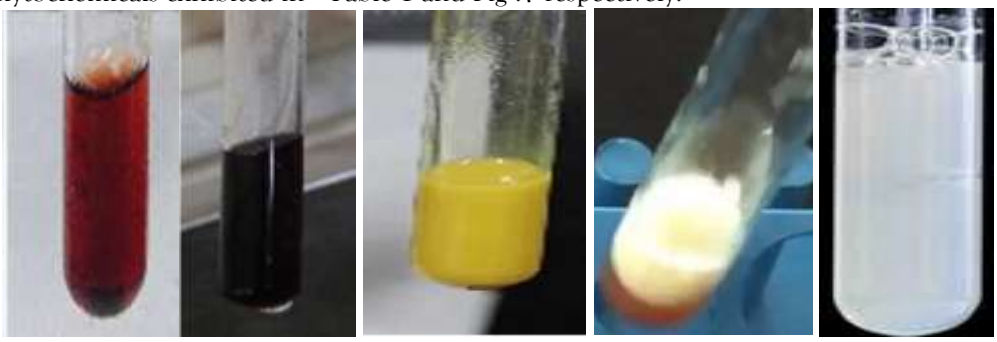


Fig 6. DLS shows silver nano particle size in solution

In solution or suspension form particle under going Brownian moment are shown on the X- axis (red), characteristic examples of a well-fit autocorrelation curve (green).The intensity of light scattering is taken as a consideration in Y-axis. The nano technology pragmatic preparation of ethnomedicine shows potent therapeutic effects. It is due to the presence of valuable phytochemicals in Ionidium Suffruticosum. In phytochemical determination alkaloid (reddish brown), Tannins (bluish black),flavonoids(yellowish), saponins(foam or froth) and phenol derivatives(white precipitate) were identified. The identified phytochemicals exhibited in Table 1 and Fig .7 respectively.



(A) **(B)** **(C)** **(D)** **(E)**

Fig.7- Phytochemical determination **(A)** Alkaloids**(B)**Tannins **(C)** Flavonoids **(D)** Saponins and **(E)** Phenolics

Table 1

Phytochemicals	**Methods**	**Observation**	**Results**
Alkaloids	Wagner's test	Reddish brown	Presence of alkaloids
Tannins	Ferric chloride test	Blackish blue	Presence of tannins
Flavonoids	Ammonia test	Yellowish	Presence of flavonoids
Saponins	Foam test	Foam formation	Presence of saponins
Phenolics	Gelatin test	White precipitate	Presence of phenolics

The bactericidal activity of silver nano particles exabits constructive result, while equated to tetracycline assayed as standard or control antibiotics . The Double distilled water, used as a negative control did not show the inhibition zone. The inhibition zone of standard antibiotics tetracycline shows 12 mm diameter(Table 2; Fig 8).The bactericidal activities of prepared silver nanoparticles counter to bacterial pathogenic strains and resulting inhibition zones were paralleled with antibiotic Tetracycline. The silvernanoparticles shown more active antimicrobial proficiency and killing activity against, Staphylococcus aureus, Klebsiella Pneumonia, Shigella and Salmonalla Typhi when comparatively analysed to the standard antibiotics.

Table 2 Butchery activity against Human pathogens

Test pathogens	Negative control (solvent only)	Positive Control (tetracycline)	Ethnomedicine
S.aureus.	Nil	18 mm	16mm
K.pnumoniae	Nil	14mm	12mm
S.typhi	Nil	16mm	14mm
Shigella	Nil	21mm	18mm

Statistical study: Mean frequency of 3 changed evaluates

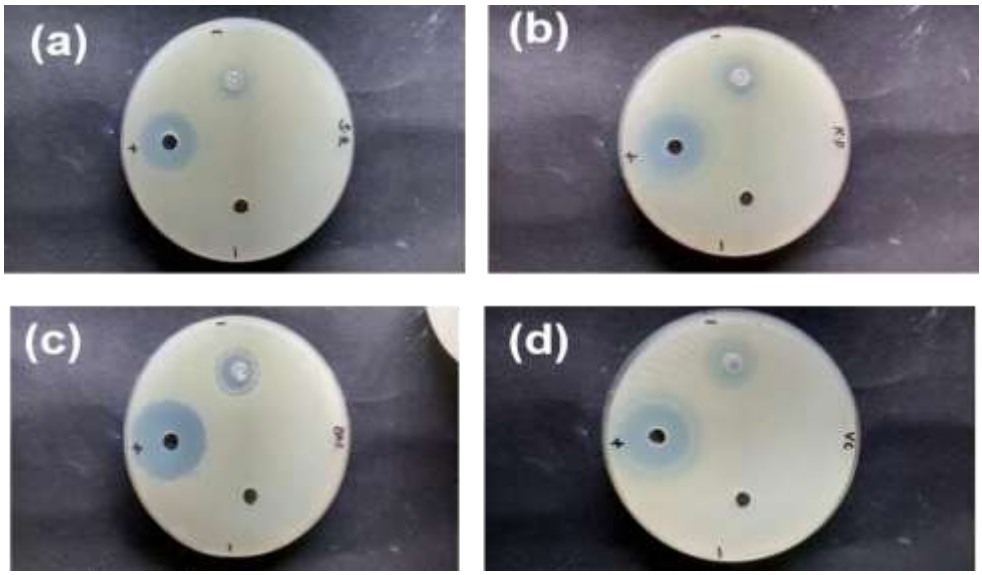


Fig 8. Bactericidal activity of silver nsnoparticles against selected multi drug resistance bacterial strains. The culture plates confirms susceptible inhibitory zones. (a) S.aureus, (b) K.pnumoniae , (c) S.typhi and (d) Shigella

4.DISCUSSION

Nanotechnology partakes noteworthy progress over the usage of assets built on nanomaterials. Plants have the capability to convert as a nanomaterials, which canister to exchanges the composition and structure [21]. Nanoparticle amalgamation by means of many plant portions for example leaf, flower, roots, and fruits [22]. The conversion of plant materials into nano material and nano ethnomedicine has develop challenging research extent owing to its immense application in numerous fields including medicine and disease control. The preparation of nanomedicine using nanotechnology and green synthesis of nanoparticles to avoiding poisonous chemicals, eco-friendly, and cost efficiency engrossed countless researchers on the way to it [23]. Usage of plant portions and its biotic activities increases the efficacy of nanoparticles. Such plant based nanoparticles advantageous in medicinal ground due its butchery activity against cancer cells, bacterial cells and also have antiviral activities. This chattels correspondingly imperative in drug delivery, diagnosis of disease and as a biomarkers [24].These properties were revealed by the silver nanoparticles prepared by us using nanotechnology.

Green route synthesis technique is a component process, to materially break down rough particles to more atomic level nano particles. Henceforth, is observed as a "top–down" method in the production of fine nano particles [25]. As effectively every one drug can be mashed to fine particles irrespective of its soluble properties in liquid based or non-liquid based solvents[26,27] The acceptable size nano medicinal particles are created after their liquified molecular status and appropriate solvents/non-solvents of the drug necessity to be prepared [28,29]. Similar vicissitudes in colour in the same way been detected in previous studies [30]. This was signified by expansion of the peak which showed the development of poly dispersed leaf nanoparticles due to slow reduction rates carried out by the phytochemical constituents.

The SEM, XRD, and DLS analysis of silver nanoparticles were seen that particles of dwarf size and spherical shapes. Similar results were attained in circumstance of different leaf extracts[31].The phytochemicals such as flavonoid, tannin being act as reducing and capping agents[32]. Neem, Banana, and Hibiscus extracts formed almost spherical nanoparticles. In this study, the bactericidal activity in

relation to antimicrobial property shown inhibition zones with all-out antibacterial activity of the prepared test silver nanoparticle sample. Results attained in preceding studies[33], also sustenance the antibacterial potential of green route synthesized silver nanoparticles..

The synthesised nanoparticles were causing killing activity associated with antibiotic resistance. The silver nanoparticles efficiently inhibited the expansion of all tested bacterial and fungal strains. The silver ions caused the cell membrane deformation. The membrane devastation is ascribed to the nano size and the spherical shape of nanoparticles, which existing a larger surface zone[34]. The nanoparticles have the properties to facilitating better superficial reactions contrary to target organisms including bacteria and fungi [35].

In the stretch that crude silver particles have coarse big size medicinal particles they cannot penetrate the pathogens cell matrix, the nano silver particles cross the bacterial and fungal cell matrix and express the noticeable antimicrobial activity by destruction of the cell membrane [36]. The same results were confirmed in the present study, due to the preparation of nanosized ethnomedicine.

5.CONCLUSION

By using Ionidium Suffruticosum plant silver nanoparticles were successfully synthesized from bio reduction of leaf biomaterial . The nanoparticles have been properly characterized by means of UV-vis spectroscopy, SEM, XRD and DLS analysis. Results represented the silver nanoparticles unchanging nature and microbial killing property, these nanoparticles may be well exploited in industrial and therapeutic purposes. However, plant based nanoparticles approval and utilization necessitate more comprehensive research. This study acquaint with nanotechnology based preparation of silver nanoparticles as antibacterial and antifungal agents. The medicinal features of the silver nanoparticles can be used as a potential forthcoming antibacterial and antifungal therapeutic. Therefore, it is suggested that upcoming studies proceeding on such procedures should be protracted to embrace in vivo mockups and medicine wellbeing researches.

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