



Research article

Physio chemical characterization & antibacterial properties of biologically synthesized silver nanoparticles from aqueous extracts of ginger

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ABSTRACT

In this work, the synthesis of stable silver nanoparticles (AgNPs) by aqueous extracts of ginger used as reducing and stabilizing agents, respectively and its antimicrobial activity against pathogenic bacteria causing urinary tract infection. After optimizing the synthesis parameters, samples were characterized by SEM, UV-Vis spectroscopy and FTIR. Biologically synthesized Ag-NPs was tested by two techniques i.e. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) determination and agar well diffusion method to study the antibacterial properties. The initial syntheses of Ag nanoparticles were characterized by UV-Vis spectrophotometer and showed the surface Plasmon resonance band at 420 nm supported the reduction of AgNO₃ to AgNP. The average diameter of the prepared nanoparticles in solution was about 40-60 nm and were spherical in shape. Analysis of FTIR showed that secondary metabolites are responsible for bio-reduction in silver nanoparticles of silver nitrate. In this study, biologically synthesized Ag-NPs also exhibited strong antibacterial activity against bacteria (*Escherichia coli*) of the clinical isolates from patients suffering from urinary tract infection, shown effective inhibitory activity against sensitive strains of *E. coli*. (Susceptible to first-line antibiotics used to treat urinary tract infections. Results confirmed this protocol as simple, rapid, eco-friendly, non-toxic and an alternative conventional physical/chemical methods. It could be used as a safer alternative to antimicrobial and antibacterial agents for further research purposes.

Keywords: Silver nanoparticles, Ginger, Antibacterial activity, UV-visible spectroscopy, SEM, FTIR, Urinary tract infection, *E. coli*

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INTRODUCTION

Matters are reconstructed at the atomic and molecular level to achieve the particle size in the range of 1-100 nm by chemical and physical processes in this technology and this development is understood as Nanoscience [1]. This field encompasses several technologies and aids in many subjects of sciences, though new applications are discovered at increasing rates in the field of nanobiotechnology [2]. A well-known application these days is the synthesis of nanoparticles using natural resources like plants and microorganisms [3].

In various applications due to their relatively large surface area to volume ratio, enhanced mechanical strength, increased reactivity, or stability in a chemical process nanoparticles have demonstrated many biomedical applications such as antifungal, antioxidant, antibacterial, and anticancer [4]. AgNPs are the most commonly used and can be synthesized using a variety of physical, chemical, and biological methods, involving chemical, heat,

electrochemical, and photochemical reduction [5]. As physical and chemical processes of synthesis are widely but these processes require many harmful chemicals as reducing agents, which are toxic for environmental. Green synthesis utilizes non-toxic and environmentally safe materials such as plant extracts, micro-organisms, and enzymes that are eco-friendly and can be an alternative to medicine applications [6].

Ginger (*Zingiber Officinale*) is a rhizome originally used as a spice plant and also as a renowned ancient medication for several diseases such as nausea, common cold, cough, vomiting, cardiac disorders, inflammation, tumors and rheumatism [7]. The mixture of honey and ginger has a high antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria. Ginger extracts show antibacterial activity against *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Staphylococcus pneumoniae*, and *Haemophilus influenza*

pathogens. In addition, the ginger extracts may contain compounds having therapeutic activity [8].

Urinary tract infections (UTI) are one of the most frequently affecting bacterial infections within the community and in hospitals. *Escherichia coli* (E. coli), the pathogen most frequently implicated in UTIs. The most commonly prescribed fluoroquinolone is Ciprofloxacin for UTIs because it's available in oral and intravenous preparations [9]. In simple acute community-acquired infections, most commonly is by *Escherichia coli* (80%) followed by *Staphylococcus saprophyticus* (10% to 15%). Resistance from ciprofloxacin is increased lately [10].

Nanoparticles containing antimicrobial substances could be considered as a new trend of antimicrobial therapeutic agents for the prevention and reduction of deterioration of pathogenic microorganisms [11]. The present work is focused on discovering a new technique for green synthesis of metal nanoparticles of ginger extract. The investigated samples were used to evaluate its antibacterial activity against a clinical pathogen E. coli causing urinary tract infection.

MATERIALS AND METHODS

For the synthesis of AgNPs, *Zingiber officinale* (commonly called as ginger) extract was used as the reducing and stabilizing agent. 20g of ginger was washed thoroughly with double distilled water and crushed in mortar and pestle into a slightly crumbly paste. Then it was transferred in a conical flask and the total volume was made up to 100ml with double distilled water. The solution was stirred on a magnetic stirrer with heating mantle for 30mins at 50-60°C and eventually, the solution was filtered using Whatman's filter paper no. 1 [12]. 10ml of 1mM concentration silver nitrate solution and 10ml of the freshly prepared ginger extract was mixed together to make up the volume ratio of 1:1. The color of the mixture prepared is yellow and then it is stirred on a magnetic stirrer for 2hrs at 70-80°C. The preparation of liquid AgNPs was identified by the color change of the solution from yellow to brown [13][14].

Physio-chemical Characterization of AgNPs

The characterization of synthesized silver nanoparticles was carried out by the following methods. Spectral analysis was carried out using a UV-VIS spectrophotometer (of MGM CRL). The spectra between 200 and 900 nm were scanned to find the absorbance peak. The functional group of biosynthesized silver nanoparticles falling in the range of 500 cm⁻¹ to 4000 cm⁻¹ was observed by FI-IR (Bruker3000 Hyperion microscope with Vertex 80 FTIR System Model). The Morphology and Topography of the synthesized nanoparticles were analyzed by scanning electron microscopy JSM-7600F fitted with an energy dispersive spectrum (EDS). In order to create an image and elemental map of AgNPs. The reduction of silver nitrate into AgNPs at various reaction times was analyzed with an atomic absorption spectrometer (AAAnalyst 400 Model).

Antibacterial Assay

The AgNPs synthesized from the ginger extract were tested for antimicrobial activity against the bacteria E. coli which were isolated from the clinically infectious specimens. 5 sensitive strains of E.coli. (Sensitive means susceptible to first-line antibiotics used to treat urinary tract infections) & 5 multidrug-resistant strains of E.coli. (Strains resistant to first-line antibiotics used to treat urinary tract infections). Cultures were collected from the microbiology laboratory of MGM Medical College & Hospital, Kamothe, Navi Mumbai. The growth obtained on the slants was used for antibacterial assay and for future use the slants were preserved in the refrigerator at 4°C.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

This technique was performed by the broth dilution method using sterile Nutrient broth to assess the antibacterial efficacy of biologically synthesized AgNPs using the ginger extract. The bacterial concentration of culture suspension was adjusted with the help of 0.5 McFarland's (1×10⁸ CFU/ ml, 0.1 OD at 635nm). A range of serial dilution from 100µl to 800µl of biologically synthesized AgNPs and solely ginger extract within the sterile nutrient broth was prepared with appropriate positive and negative controls. All tubes accept the negative control, 100µl of standardized test culture suspension was added, and also the steps were performed under sterile conditions. The complete set was incubated at 37°C for 24hrs. And to verify the MIC and MBC results, a loopful of all the dilutions was streaked on sterile Mueller-Hinton agar plates with a sterile Nichrome wire loop and incubated at 37°C for 24hrs [15][16].

Agar-Well Diffusion Method

0.1ml of the standardized bacterial test culture suspension is spread over the sterile Mueller-Hinton agar plates to obtain a lawn growth. Wells were bored using a sterile cork borer in the test culture inoculated plates and different concentrations such as 25µl, 50µl, 75µl and 100µl of AgNPs and control ginger added to the wells with the help of micropipette. All the steps were carried out under sterile conditions. The plates were incubated at 37°C for 24hrs. The inhibition of the test culture will be visible in the form of clearance around the wells and this zone of clearance is measured in millimeter (mm) [17]. The experiments were carried out in triplicate for confirmation of the results.

Statistical Analysis

Kruskal-Wallis Test will be done for the statistical analysis of this research study. Significance of all the statistical tests is predetermined at P≤ 0.05 using SPSS (statistical package for social science) version 19 software. Statistically, the data was evaluated using the Kruskal-Wallis Test between control and test groups. It is represented as the level of significance and was considered to be P<0.05.

This color change from yellow to brown is the visual indication of the formation of AgNPs. As time passes the yellow-colored solution turns dark brown by 2hrs, which can be because of the indicating the reduction of Ag⁺ ions to AgNPs, increased concentration of NPs, and also the particle size. According to reports by Lalitha et al. and Hyllested et al. color change is an important factor for the synthesis of AgNPs.

No significant change observed after 2hrs of stirring resulting in the complete reduction reaction [18][19]. Change in the color was similar in most of the AgNPs which appear brown in aqueous medium as a result of surface Plasmon vibrations as shown in the study of Otunola GA et al. [19].

Optical Characterization

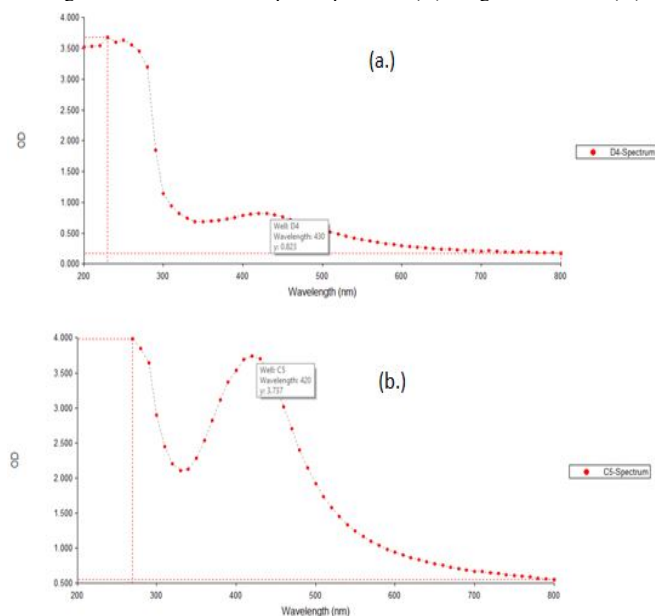
UV Visible Spectroscopic Measurement

According to the study of S Mahadevan et al., UV Visible spectroscopy is one of the most important techniques which can confirm the prepared materials are nanoparticles [20]. The range of broad absorption spectrum between 200 and 800 nm and the exact peak occurred at 420 which was indicative of the peak of silver nanoparticles. Fig.1 shows the spectroscopic analysis of synthesized silver nanomaterials using the ginger extract. The absorption peak (λ_{max}) is visible at 430 nm and the optical density is 0.823 of ginger extract. The intensity of the absorption band increases with an increasing period of time of aqueous component and consequent color changes were observed from yellow to reddish-brown, shown in Fig 1.

With time the presence of the surface resonance plasmon (SPR) band increased in size. According to the peculiar optical properties of silver nanoparticles, the study of the spectra also obtains an excellent amount of data about the physical state. The spectra clearly show the increase in silver solution intensity over time, suggesting the development of an increased number of AgNPs in the solution. The sharp bands of silver colloids were observed at ~420 nm and the optical density is 3.737 for AgNPs synthesized using ginger extract at 70-80°C for 2hrs and similar results were obtained in the study performed by S Ahmed et al. [3].

The optimum temperature required for the completion of the reaction was investigated to be 70-80°C for 2hrs. Upon a further increase in temperature (up to 85°C), no further absorbance increase was observed and shows an increase in AgNPs size. Further increase in temperature caused the broadening of the peak revealing the increased size of nanoparticles. This temperature-dependent increase within the peak intensity demonstrated the reaction temperature dependence of the silver ion reduction. The reduced concentration of silver ions has been observed to increase by rising temperatures. Here, ginger is also acting as a stabilizing agent as the readings of AgNPs were taken for 3months and the results were found to be constant.

Figure 1. UV-visible absorption spectra of (a.) Ginger extract and (b.)

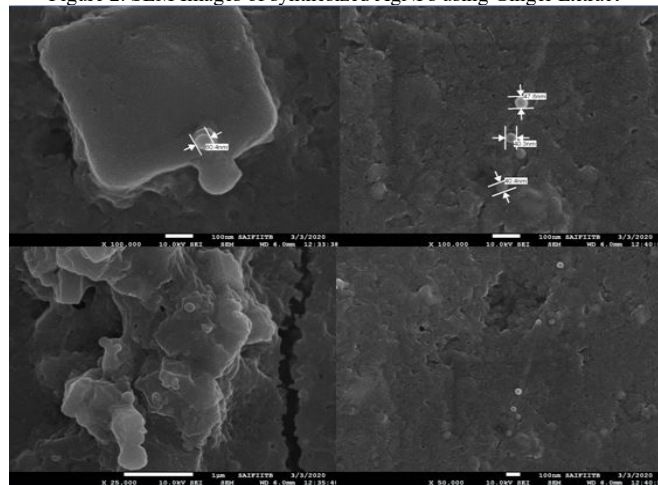


AgNPs synthesized using 1mM silver nitrate solution and ginger extract in 1:1 ratio and stirred at 70-80°C for 2hrs

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was employed for the surface morphology of synthesized NPs. The scanning electron micrograph at 100,000x of AgNPs synthesized using ginger is presented in Fig 2. The different size range (40-60 nm) of AgNPs is clearly illustrated by SEM images. Furthermore, the scanning electron micrograph revealed that most of the particles were spherical in shape. Some nanoparticles size is larger because silver nanoparticles have the tendency to agglomerate due to their high surface energy and high surface tension of the ultrafine nanoparticles. Few particles were also found to form small aggregates which can be due to agglomeration or improper capping.

Figure 2. SEM Images of synthesized AgNPs using Ginger Extract

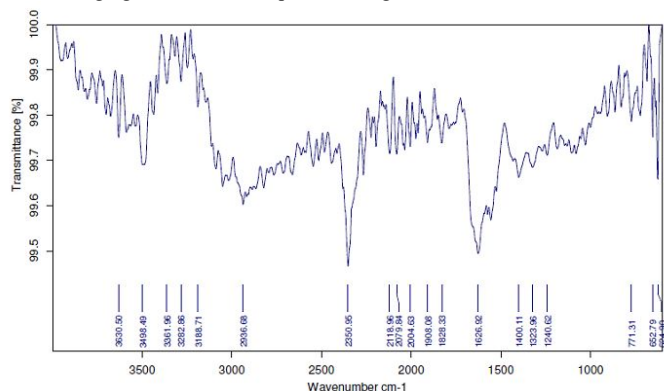


Fourier Transform Infrared Spectroscopy (FTIR)

Following on from Sasidharan et al. FTIR has proven to be a valuable resource for characterizing and distinguishing compounds or functional groups, and pure compound spectrums are typically so distinctive that they are like a molecular "fingerprint". The FTIR

analysis revealed different stretches of bonds at different peaks for ginger extract. Strong and sharp peaks in the range of 4000-3000 cm^{-1} are due to O-H and N-H stretching of free alcohols and the primary and secondary aliphatic amines also medium sharp peaks in the same range is due to O-H and C-H stretching of carboxylic acids, alkynes, and alkenes. In the range of 3000-2000 cm^{-1} absorption, peaks were observed due to O=C=O stretching of carbon dioxide, C \equiv C, and C=C=O stretching of alkynes, ketones, and nitrites (Fig:3). In this study, the bands observed denote stretching vibrations responsible for compounds such as flavonoids, phenols, terpenoids, and proteins. Absorption peaks in the range of 2000-500 cm^{-1} are due to C-H, O-H bendings and C-N, C=C stretching's of aromatic compounds, alcohols, phenols, amines, and alkenes-H stretching are of amide linkages in the proteins of ginger and these results of FTIR prove the presence of proteins in the biosynthesized AgNPs as coat coverings and are called capping proteins which prevent agglomeration and maintains the stabilization of AgNPs. and could confirm that these biomolecules in the extracts were responsible for reducing, capping, and stabilizing of the AgNPs with the studies performed by N Ahmad et al. [21].

Figure 3. FTIR spectral analysis of the biologically synthesized AgNPs using ginger extract in the spectrum range of 4000 – 600 cm^{-1}



Atomic Absorption Spectroscopy (AAS)

By calculative estimations, the concentration of silver incorporated initially before synthesis is 0.0001698gms/ml for 1mM of silver which comes to 160ppm. After adding the ginger extract to the reaction mixture and stirring at 70-80°C for the reaction period of 2hrs. The product solution was analyzed by atomic absorption spectroscopy and results were found to 34.44ppm indicating a decrease in Ag⁺ ions and also the reduction of Ag⁺ ions into AgNPs.

YANG Nan et. Al., the study is quite similar though except for a few changes in the protocol. But the results obtained were comparable, such as the color of the solution changed from yellow to brown after the reaction and the absorption peak was found to be between 400-450nm for UV spectrophotometer analysis. By SEM analysis the size range of the AgNPs is 40-60nm and morphology is distinctly spherical in shape and the FTIR results showed similar

absorption peaks representing the presence of similar functional groups [17].

MIC and MBC determination

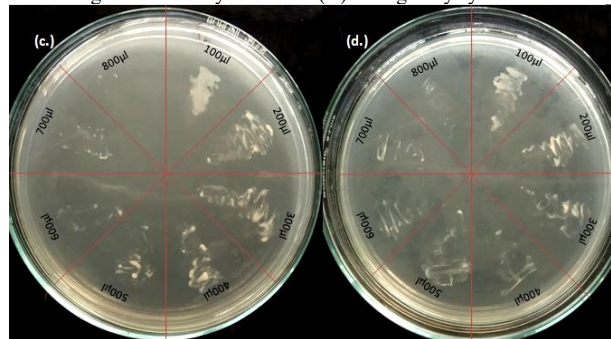
Silver nanoparticles are well recognized as the most common antimicrobial substances due to their strong biocidal impact on microorganisms, which have been used for the prevention and treatment of various diseases in recent decades. AgNPs are also widely used as anti-fungal, anti-inflammatory and anti-viral properties. Recently, non-hazardous AgNPs can easily be synthesized using a cost-effective method and tested as a new type of antimicrobial agent. Ginger AgNPs exhibited the maximum inhibition for both Gram-positive and Gram-negative bacteria. In this study, the application of biologically synthesized AgNPs as an antimicrobial agent was tested against selected Gram-negative bacteria on an agar plate and liquid medium. The results showed that the tested bacteria could completely inhibit by AgNPs.

The MIC of ginger extract for sensitive and resistant strains of E.coli do not fall in the standardized range 100-700 μl and which was considered to be above 800 μl . Whereas the MIC of synthesized AgNPs for sensitive strains of E.coli is 500 μl and for the resistant strains is 800 μl . For other experiments, MIC and MBC are carried out with solid nanoparticles or by drying the liquid nanoparticles and perform the process of the broth dilution method. Our study was in a similar line with Sri Ramkumar Vijayan et. Al in their study MIC of liquid nanoparticles in the range, 5-100 μl was performed [16].

Viability Test

MIC of ginger extract for both sensitive and resistant strain of E.coli is above 800 μl . The MBC of synthesized AgNPs for the sensitive strain is 800 μl and for resistant strain, the MBC does fall in this range and so it is considered to be above 800 μl . These results were found by the broth dilution method and confirmed by the viability test. As the previous study by Guzman et al. (2012), reported that AgNPs employed antibacterial activity on Gram-negative bacteria [22].

Figure4. Viability test of set (c.) biologically synthesized



AgNPs using ginger extract on sensitive strain of E.coli and set (d.) MDR strain of E.coli causing urinary tract infection.

The significance of the statistical test was predetermined at $P \leq 0.05$ using SPSS (statistical package for social science) version 19 software. The p-value calculated with the help of the Kruskal-Wallis test was

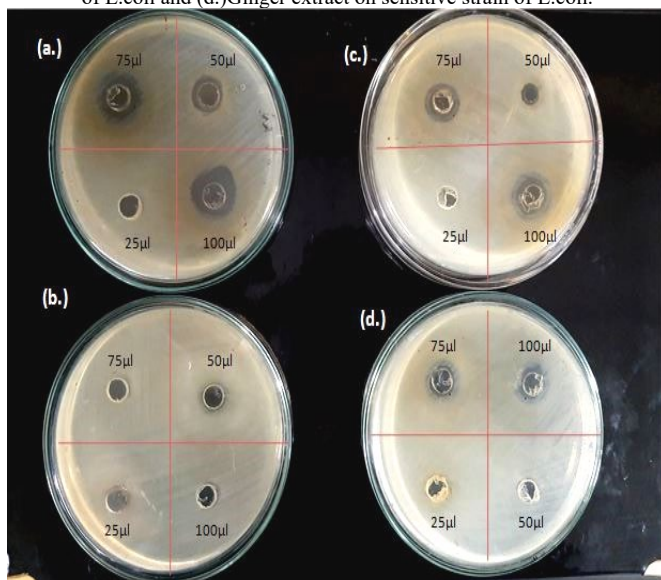
found to be 0.00921 (Fig 4). So, this proves there is a significant difference between both the groups i.e. Ginger extract and synthesized AgNPs using the ginger extract.

Agar Well Diffusion Method

To determine the antibacterial effect, standard microbiological tests such as agar diffusion and MIC have been widely used. The agar diffusion method in which microbial inhibition zone depends on the solubility and infusibility of the test material and, thus, its maximum potential may not be exhibited. Direct or close interaction between the microorganism and the samples is measured by direct contact tests, regardless of the diffusion properties of the material and media being tested, which is an advantage over the agar well diffusion method such as MIC [23].

The maximum zone of inhibition obtained by ginger extract for the sensitive strain is 11mm by 100µl concentration and no inhibition is observed for the resistant strain of E.coli. Antibacterial activity by the synthesized AgNPs for the sensitive strain was more compared to the resistant strain of E.Coli. The biggest zone of inhibition was observed by synthesized AgNPs on the sensitive strain of E.coli with 17mm by 100µl concentration. In the research work of Shaheen Sheikh et. Al. green synthesis of AgNPs was carried out using several plants, one of which was ginger, and its antibacterial activity was tested on E.coli clinical isolates causing urinary tract infection. The inhibition zone for 100µl of AgNPs was 12 mm [24], whereas, according to our research, biologically synthesized AgNPs tested on sensitive *E.coli* strain showed 17 mm clearance by 100µl concentration, and for the same concentration, *E.coli* MDR strain received 13 mm clear zone. The results from this study shows that plant extract AgNPs exhibit enhanced broad-spectrum antibacterial activities. This suggests that nanoparticles from ginger extracts could yield valuable alternative as antibacterial drugs.

Figure 5. Agar well diffusion assay of set (a.)AgNPs on sensitive strain of E.coli, (b.)Ginger extract on MDR strain of E.coli, (c.)AgNPs on MDR strain of E.coli and (d.)Ginger extract on sensitive strain of E.coli.



CONCLUSION

In this study, an eco-friendly method for the synthesis of AgNPs using the aqueous extract of ginger was developed. Characterization AgNPs revealed that the particles were spheroidal in shape with a particle size distribution range of 40-60 nm. The AgNPs in solution is relatively stable. The synthesized AgNPs have demonstrated potential antibacterial activity against both pathogenic strains of E.coli causing urinary tract infection and are verified by the techniques of antibacterial assay i.e. MIC & MBC, and agar well diffusion method. It is evident that the synthesized AgNPs can be implemented for their use as antibacterial agents for further research purposes.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript.

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This study has not received any external funding.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests.

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