Green Synthesis, Characterization and Biological Activity of Silver Nanoparticles Using Ruta Leaf Extract

Green Synthesis, Characterization and Biological Activity of Silver Nanoparticles Using Ruta Leaf Extract

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Abstract. In this paper, green synthesis of AgNPs is carried out by means of an environmentally friendly, cost-effective and safe technology using Ruta leaf extract as a reducing agent for silver. Furthermore, biological activity of produced AgNPs was examined against different microorganism with multiple resistances to antibiotics, which isolated from different disease states. The produced AgNPs were characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Field Emission Scanning electron microscopy (FESEM) and Dynamic light scattering (DLS). The biosynthesized silver nanoparticles by Ruta leaf extract were confirmed by their change of color from colorless to yellow and lastly to reddish brown. UV-visible spectrophotometric analysis of the AgNPs synthesized showed characteristic surface plasmon absorption peak at 415 nm. Where the X-ray diffraction showed that, the nanoparticles were crystalline in nature with a face-centered cubic structure (FCC). Field Emission scanning electron microscopy (FESEM) micrograph showed the creation of spherical nanoparticles with size from 30 to 50 nm in diameter. The element silver of the sample was obtained from the Energy Dispersive Analysis of X-ray (EDX). The stability and distribution size were detected using zeta potential and DLS analysis.

Keywords: Silver nanoparticles, XRD, EDX, FESM, Antibacterial, Antifungal.

1. Introduction

In last years, nanoscience has been widely deliberated and the speedily increasing technology of utilizing and producing nano-sized particles, because of its biological applications and unique properties [1]. Nanoparticdes can be manufactured by different methods such as physical, biological and chemical methods [2]. Both chemical and physical processes for production of nanoparticles comprise pyrolysis, laser ablation, physical or chemical vapour deposition, lithography electro-deposition, Sol-Gel and nearly all of them have toxic influences on the health of human, that restricts their enormous Application [3, 4]. As for the biological preparation of nanoparticles, it is careful a clean, environmentally friendly and non-toxic method compared to chemical and physical ways [5].

There are various kinds of metal inorganic nanoparticles for example (Silver, Zinc, Copper, Gold, Titanium, Nickel and Magnetite) are manufactured by several kinds of the plants, where the vegetable extracts doing as capping and reducing agents also is result in the creation of the crystalline nanoparticles with a diversity of Forms and the Sizes between (1-100) nm [6]. Through several inorganic nanoparticles, AgNPs are give distinct importance and draw additional consideration of seekers due to it's several application in the sites of bimolecular diagnostics [7], catalysis, therapeutics, micro-electronics [1]. In addition, the researches have also exposed that the medical use of silver nanoparticles comprises it's use as antiseptic [8], anti-inflammatory, antimicrobial, antioxidant agents, anti-diabetic [1] and also reported in the cancer diagnosis and treatment [9].
The properties of silver nanoparticles synthesized using the green method depend on several factors, including the temperature, nature and concentration of the plant extract, the pH, the mineral concentration, and the contact time between the extract and the silver nitrate ($AgNO_3$) [10].
Plant Ruta (Rutaceae), famous in certain countries as Sazab and has a known term as rue, is extensively in deferent topographical areas of Afro Asian countries [11]. This herb is considered as an ornamental plant [12] and is applied in customary medicine for handling several disorders for instance hypertension, edema, Hysteria contractions, skin conditions, worms, womb diseases and digestive disorders [11, 12]
The purpose of this research is production of silver nanoparticles (AgNPs) using Ruta leaf extract, as well as to study the bio-activity of silver nanoparticles as an anti-bacterial such as (Staphylococcus aureus) and an anti-fungicide (Candida albicans)

2. Materials and Methods

2.1. Bacterial and Fungal Isolates

A type of bacteria, Staphylococcus aureus, was obtained from Ramadi Teaching Hospital in Iraq. A species of fungi namely Candida albicans was gotten from the College of Science at Baghdad University, Iraq.

2.2. Collecting and Preparing Plant Extract

The leaves of the Ruta plant were collected from a farm in Kabisa sub-district in Heet city in Iraq. It was cleaned of dust with washed well with distilled water, then with deionized water and then placed in an electric oven for drying, taking into account the constant stirring. After the leaves dried, they were crushed by an electric grinder and put in a closed box and kept at room temperature for more use. 3 g of the ground material powder was boiled with 150 mL of deionized water in a 250 mL conical flask, at 90 °C for 20 minutes, the leaf extract was chilled and filtered through Whitman filter paper, and the plant extract was stored at 4 °C in the refrigerator until use.

2.3. Preparation of AgNPs

The basis of silver is silver nitrate ($AgNO_3$) in deionized water. An aqueous solution of 20 mL, 1mM $AgNO_3$ was added to 1mL of Ruta extract and the mixture was stirred with a magnetic stirrer at 90 °C. The solution color was changed from the colorless to dark brown. Manufacture of AgNPs was primarily known by color change and it's synthesis was complete using UV-Vis analysis.

2.4. Characterization of Synthesized AgNPs

The formation of AgNPS was periodically monitored by visual observation and measuring the UV-Visible spectrum. In visual observation, color alteration of the solution was certain by naked eye. Silver nanoparticles in solution were recognized by using UV-visible spectrometer (INOVIALAB, UV-visible 1911 DB spectrometer, China) in the wavelength (A) range (300-700) nm. Surface chemistry of silver nanoparticles and biomolecules in Ruta extract were characterized by using Fourier transform infrared spectroscopy (FTIR) (BRUKER ALPHA FTIR spectrophotometer, Platinum ATR). The FTIR spectra were registered from (4000-400) cm^{-1}. The phase distribution, crystallinity and purity of the produced AgNPs was proven by X-ray diffraction (XRD) (SHIMADZU Japan, XRD

600). The dynamic light scattering technique was used to analyze the particle size and zeta potential of the silver nanoparticles using (DLS) (Brookhaven, UK) size range (1-10000) nm. The size, surface and shape of synthesized AgNPs were studied by Scanning Electron Microscope Filed Emission (FESEM) (TESCAN MIRA3 FEG-SEM, Australia), with EDX analysis (Oxford) was also used for the analysis.

2.5. Antimicrobial Assay of AgNPs

The antibacterial activity of AgNPs was scrutinized using agar well diffusion test [13]. The tested bacteria were mopped regularly on nutrient agar plates by disinfected cotton swab and four wells of (5 mm) width were made by sterile well borer 70 µL of silver nanoparticles solutions with several concentrations (1, 2, 5 and 10) mM was poured into the corresponding well.

Antifungal property of the AgNPs was also tried against Candida albicans by agar well diffusion method [13]. The C. albicans suspension was mopped on the sabouraud dextrose agar dishes. The 5 mm diameter wells were hole on the surface of the sabouraud dextrose agar plates. Each well was full with 70 µL of the AgNps solutions with several concentrations (1, 2, 5, and 10) mM. The dishes were incubated by 37 °C for 24 h for the yeast and bacterial isolates. Then, the distance of damping zone was measured, and compared by the inhibition zone of (the gentamicin and nystatin) disc as a standard positive control drugs against bacteria and fungi respectively.

3. Results and Discussion

3.1. Visual Observation of Silver Nanoparticles

The production of silver nanoparticles begins as soon as ruta extract is introduced into a 1mM of $AgNO_3$ solution. The continuing alteration in the color of the liquid from uncolored to yellow and lastly to reddish brown designates the creation AgNPs of as shown in Figure 1. This color alteration is due to the characteristic of Surface Plasmon Resonance [14] an visual property distinctive to the noble metals, which indicates the creation of silver nanoparticles, due to the reduction of the silver metal ions Ag^{+} to the silver nanoparticles Ag° [14]. This is due to the active elements present in Ruta plant extract.

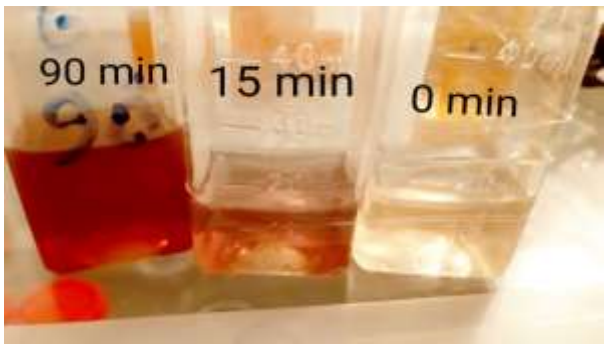

Figure 1. Visual observation at 0 min, 15 min and 90 minutes.

3.2. UV-Vis Spectroscopy

UV- Visible Spectroscopy is the primary way for determining the presence of silver nanoparticles through green synthesis [15]. The synthesis of silver nanoparticles in solution was additional definite by UV-Vis analysis. The wavelength was measured in a range between 300 and 700 nm. The maximum absorption is observed at 415 nm Due to surface plasmon resonance as in Fig. 2 which is characteristic of AgNPs [15].

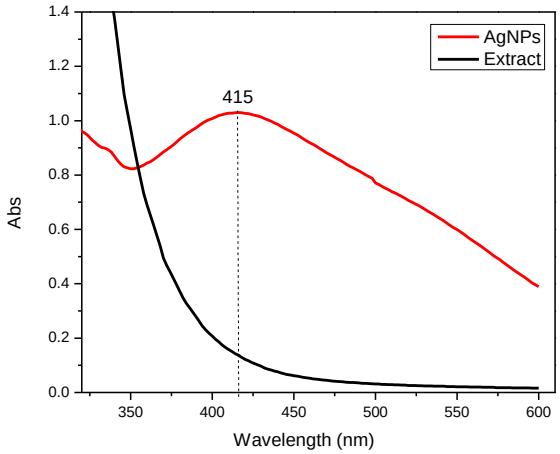

Figure 2. UV–Visible absorption spectra of AgNPs.

3.3. X-ray Diffraction Spectroscopy

X-ray diffraction (XRD) technique can be used to confirm the existence of AgNPs and to investigate the crystallization of AgNPs. The crystal size (D) of the nanoparticles is estimated by the flowing Scherer - Debye equation [16]

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

where: (D) is crystal size, (K) is a constant equal to 0.9, (λ) is the X-ray wavelength used 0.15406 nm, (β) is the values transformed (FWHM) from (degree) to the radial angle by multiplying it by (π /180) and θ is the Bragg angle for the diffraction. While the dislocation density (δ) was calculated by equation [17]

$$\delta = \frac{1}{D^2} \quad (2)$$

The micro strain (Ɛ) of lattice was calculated by equation [18]

$$\varepsilon = \frac{\beta_{1/2}}{4 \tan\theta} \quad (3)$$

The XRD results showed in Fig. 3 that the silver nanoparticles AgNPs are in crystalline nature with faceted centered cubic crystal structure (FCC) attributable to the silver nanostructure according to standard metal silver XRD pattern JCPDS No. 04-0873. The peak of (111) has the highest intensity which indicates that (111) is the preferred orientation is consistent with previous studies [18]. In adding to the Bragg peak characteristic of silver nanocrystals, extra peaks were also observed at 30.90° and 32.15° where these peaks are owing to the phytochemical elements, which are existing in the extract and accountable for silver nitrate ($AgNO_3$) reduction, and stabilization of resultant silver nanoparticles [19, 20]. The microstructural parameters and crystal sizes of the AgNPs are logged in Table 1.

Table 1. The microstructral parameters and crystal size of AgNPs.

2θ(°)	*Plan (hkl)*	*d-spacing (Å)*	*FWHM (β)*	*Crystallite Size (D) (nm)*	Dislocation Density $(\delta) \times 10^{11} / cm^2$	Micro Strain $(\varepsilon) \times 10^{-3}$
38.01	(111)	2.36	0.65	12.63	6.269	8.235

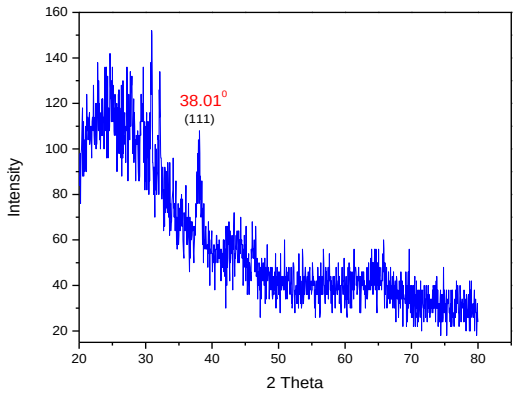

Figure 3. XRD pattern of AgNPs produced using by the leaf extract of Ruta.

3.4. FTIR of The extract and it's AgNPs

The extracts were spectrophotometrically distinguished using the infrared spectrum (FT-IR) to find out the chemical composition and the active groups present. We note the similarity in the sites of the beams and significantly where the infrared spectrum of the extract showed Fig. 14 (a) the presence of an absorption beam at 1637cm^{-1} due to the stretching quivering of the group (C=O). It refers to the carbonyl present in the composition of proteins, amino acids, peptides and flavonoids. Also, which confirms that the two extracts contain flavonoids, proteins, amino acids, peptides, polyphenols and sugars is the presence of an elastic shaking of the absorption beam at 3253 cm^{-1} and the absorption beam at 580 cm^{-1} indicates the existence of an amide group (O=C-N-H) which It binds two successive amino acids in the conformation of peptides or proteins and the absorption bundle at (3150-3350) cm^{-1}, which is supposed to be separated in two bundles from the bottom, designates the presence of the group (-NH_2) in the composition of proteins and amino acids. As for the evidence of the presence of the carboxyl group (-COOH) in peptides, amino acids and proteins is the emergence of a wide absorption band with a range (2520-3600) cm^{-1}, as well as the presence of the carboxyl group (-COOH) in monosaccharides [21-26].

Fig. 4 (b) shows the FTIR spectrum of nanoparticle with extract, it was noticed that, in general, this spectrum is actual similar to the infrared spectrum of the regular Ruta plant, and thus the chemical composition is very similar except for the new clear beam at 1075 cm^{-1}, which is due to the returning CO For phenols, carboxylic acids, amines, peptides, and proteins, the reason for this clarity is the presence of nan atoms of silver with good diffusion and with a surface area, the dynamic groups in the nano extract, which are rich in electrons, can participate with their electrons in the empty orbital in the outer shell of silver, where that silver atoms comprise 47 electrons, its fifth envelope contains single electron in the secondary level ((5S) and thus the remaining secondary levels (5p) comprising three orbitals, (5d) having five orbitals, and (5f) comprising seven empty orbitals, it can house the electron pairs coming from good electrical properties, negative atoms, for example, the oxygen atom (O), which is present in the composition of sugars in the form of (-OH) and in the flavonoids in the form of ether (-O-) or in the form of carbonyl (C=O) in the aforementioned compounds, so the link is as follows (Ag-OR), where R is an amino acid sugar, a polypeptide, protein, multiple phenols or

flavonoids and another example of atoms with good electrophoresis, it is the nitrogen atom (N) in the composition of amino acids in the form of ($-NH_2$) or (=NH) as in arginine and in peptides and proteins in the form ($-NH_2$) and in the form (-NH-C=O) so the link is as follows (Ag-NR) where R is an amino acid, peptide, or protein [17, 18, 27, 28, 29 and 30].

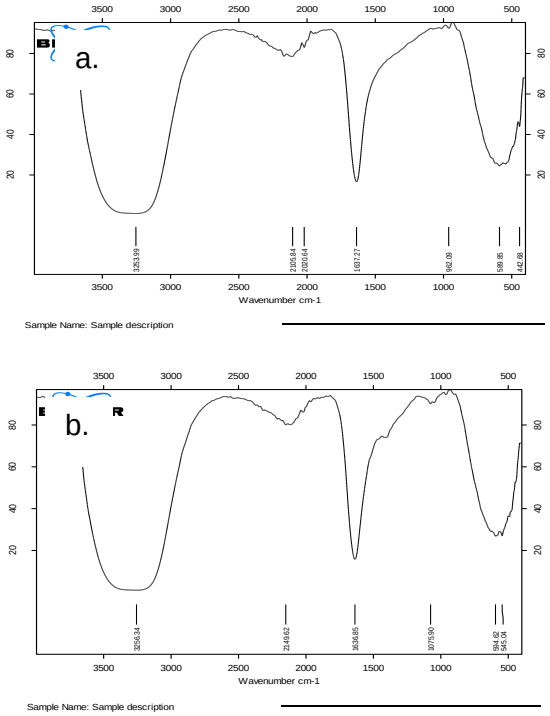

Figure 4. FTIR spectrum of: (a) Ruta extract, (b) Ruta extract with AgNPs.

3.5. FESEM with EDX Analysis

The morphology of the silver nanoparticles was examined by Field emission scanning electron microscopy. The FESEM images of the AgNPs are exposed in Fig. 5. The surface morphology of AgNPs exposed spherical nature and even shape. In the current analysis, the graph of the particle size ranges from 30 to 50 nm. As results are agreement with studies for phyto- produced silver nanoparticles [31]. This upshot confirms that Ruta extracts might action as a capping agent and reducing in the fabrication of AgNPs.

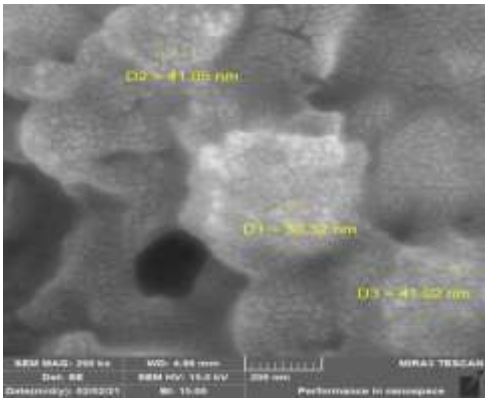

Figure 5. FESEM image AgNPs synthesized using the leaf extract of Ruta**.**

Fig. 6 shows the quantitative info of biosynthesized AgNPs, which indicates the existence of silver as the component element. Metal silver nanoparticles display a naturally strong indication peak at 3 keV, because of surface plasmon resonance [32]. Appearance of other peaks (Si, Na and Ca) was presumably linked with the glass beneath, which carries the sample [33] and the attendance of elements such as C, O, Au, Mg and Cl, are one of the characteristic of nanoparticles produced by plant extracts [32].

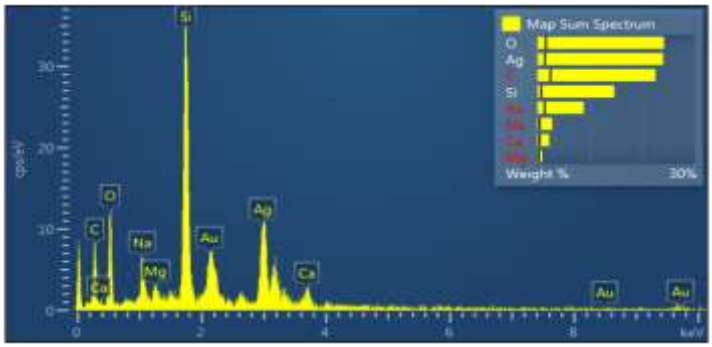

Figure 6. EDX of AgNPs synthesized using the leaf extract of Ruta**.**

3.6. Particle Size and Zeta Potential for the AgNPs

The particle size distribution curve of silver nanoparticles is shown in Fig. 7. The dynamic light scattering size distribution (DLS) graph shows that the average size of these AgNPs is 89 nm. The distribution in the lower particle size range indicates that the manufactured particles are also in the lower particle size range.

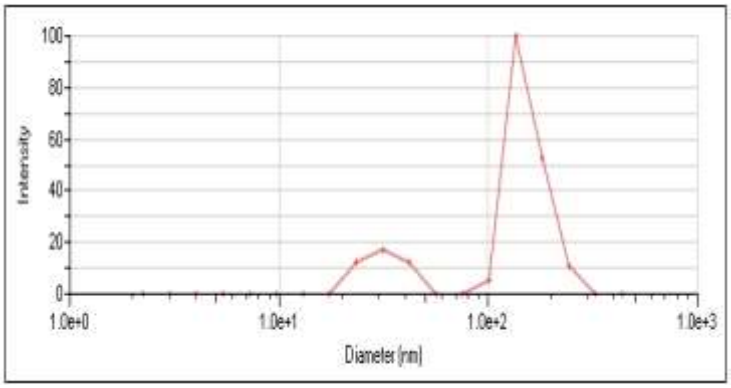

Figure 7. DLS of AgNPs produced using the leaf extract of Ruta**.**

The zeta potential value was determined as −22.53 mV (Fig. 13). It is proposed that the surface of these silver nanoparticles (AgNPs) is negatively charged. The value of negative the nanoparticles confirms the repulsion between them and proves that they are very stable, which indicates the stability of the nanoparticles and their non-clumping [34].

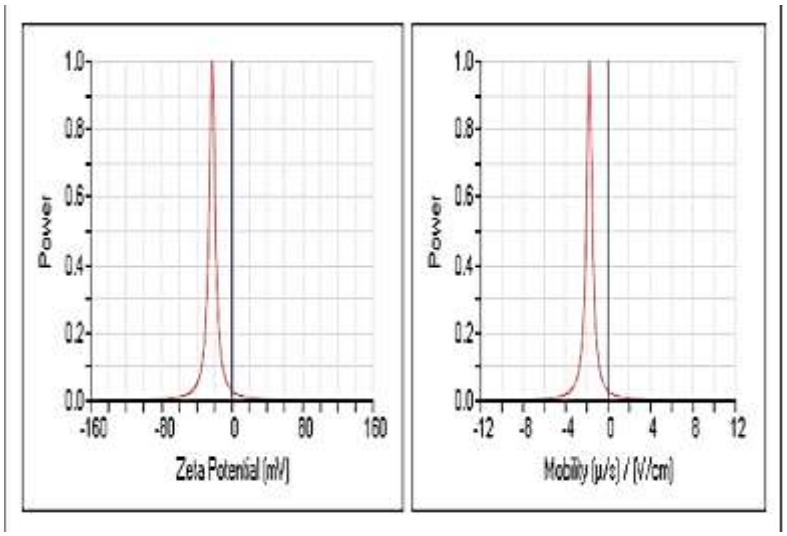

Figure 8. Zeta potential for AgNPs produced using the leaf extract of Ruta**.**

3.7. Antimicroorganisms Activity of AgNPs

A silver Nanoparticles exposed antimicroorganisms work against examined Pathogenic Microorganism, with shifting degrees, as proposed by the width across of inhibition zone, Fig. 9 (a). The antimFungi work against C. albicans appeared bigger zones inhibition, In comparison to the gram-positive bacteria (S. aureus), Fig. 9 (b), which may because of the difference in cell wall structure. The Gram-positive bacteria cell wall consisted of a dense peptidoglycan layer, involving linear polysaccharide chains cross related by small peptides, hence constructing additional rigid structure result in the problematic penetration of silver nanoparticles [35]. From the results, we note that a surge in the concentration of Silver nitrate increases the area of inhibition, due to the surge in the quantity of silver ions, which results in an amplified biocide effect, which depends on the dose and size.

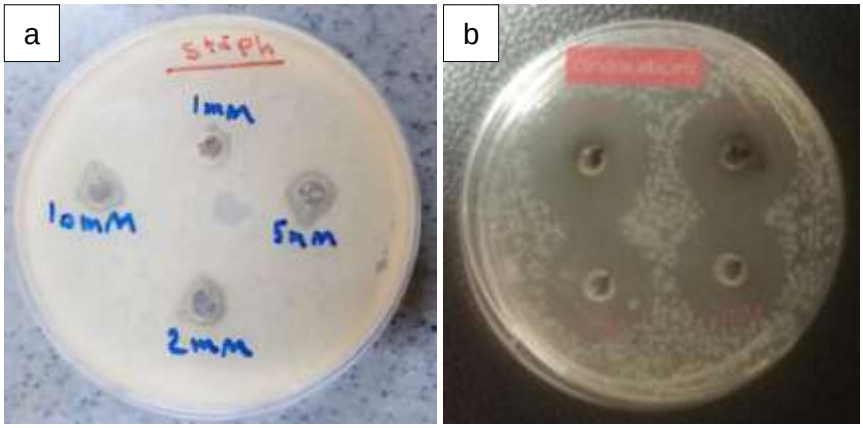

Figure 9. Zone of inhibition of silver nanoparticles against: (a) S. aureus, (b) C. albicans**.**

A gentamicin and nystatin was used as the positive control drugs against bacteria and fungi respectively. The inhibition zones were measured and compared by the inhibition zone of (the gentamycin and nystatin) disc as a standard positive control Table 2.

Table 2. Antimicrobial activity of (AgNPs) at altered concentrations.

Microbial Isolates	***Diameter of inhibition zone (mm) AgNPs at different concentrations***				***Positive Control***
	1 mM	2 mM	5 mM	10 mM	
S. aureus	7	10	11	12	13
C. albicans	26	28	31	30	14

Numerous chief mechanisms underlie the biological characteristics of (AgNPs) in working against microbes (1) AgNPs can reason more risk to microbial cells by permeating the cell, by they interact with proteins DNA and other cell components that contain phosphorous and sulfur. (2) AgNPs liberationsilver ions, producing an amplified biocidal effect, which is dose and size -dependent (3) AgNPs Stick up to the cell surface is negatively charged, causing its chemical and physical properties to change of the cell wall and the cell membranes and disturb significant functions such as osmoregulation, permeability, respiration and electron transport [14, 36].

4. Conclusion

In conclusion, it has been demonstrated that rota leaf extract is capable of synthesizing silver nanoparticles. It is a fast, environmentally friendly biosynthesis and a simple and effective way to synthesize nanoparticles. These gotten silver nanoparticles have potential applications in the biomedical field and this simple procedure has many advantages such as compatibility with pharmaceutical and medical applications and as cost effective. The bioavailable silver nanoparticles showed excellent antimicrobial activity.

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