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Research Article

Green Synthesis of Gold and Silver Nanoparticles Using *Opuntia dillenii* Aqueous Extracts: Characterization and Their Antimicrobial Assessment

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In the present study, gold (Au) and silver (Ag) nanoparticles (NPs) were synthesized from aqueous extracts of *Opuntia dillenii*, characterized by various advanced techniques and investigated for antibacterial and antifungal potential. Phytochemical screening of *O. dillenii* showed the presence of alkaloids, betacyanin, saponins, tannins, flavonoids, and phlobatannins. The characterizations of the synthesized metal NPs were performed such as UV-visible spectrophotometer, FTIR (Fourier transform infrared) spectrophotometer, SEM (scanning electron microscopy) and EDX (energy-dispersive X-ray). Through the application of such advance techniques, the UV-visible spectrophotometer showed the bands of absorbance for AgNPs and AuNPs at 420 nm and 525 nm range, respectively. The FTIR spectra for both and AgNPs also appeared in the range of 4000-400 cm^{-1}. SEM was performed for the textural and morphological characteristics of the NPs such as shape, size distribution, and surface structure. Elemental analysis was recorded for the synthesis of Au and AgNPs, which confirmed its purity. The *O. dillenii* extract and their synthesized Au and AgNPs showed a clear zone of inhibition against the *E. coli*, *S. aureus*, *K. pneumonia*, and *P. aeruginosa* bacteria and *T. viride*, *C. albicans*, *C. krusei*, and *A. niger* fungal species.

1. Introduction

The past decades have witnessed rising importance of nanotechnology in medicine and healthcare [1–3]. Keeping this in view, the green synthesis methods which are having abilities of producing the environment friendly nanoparticles NPs are being adapted by the various field experts [4]. Therefore, the importance is being given to such technologies which are not only environment friendly but also have the wide range of the nutrients. The NPs are crystallographic with high surface area and they are very small sized structures having high reactivity [5]. So, the use of green technology is now becoming more popular than ever before.

The crops at subtropical areas are difficult to grow because of the lack of water and salinity in soil. The natural beauty of the subtropical areas may be enhanced by using the species of the *Opuntia* which may be grown in the

subtropical areas. *Opuntia* is a genus which is medically important as well and it is required to be explored in term of the medical characteristics. *Opuntia* specie was first grown in America as there are many subtropical areas in America where this *Opuntia* specie may be easily grown. It is mostly found in dry and scarce water condition as it is a xerophytic plant. Specific species including *O. dillenii* belongs to the cactus family and is commonly called as prickly pear plant, with 1500 known species across the globe [6]. Hence, the scholars emphasized on cultivation of the *O. dillenii* in the tropical and subtropical areas for the purpose of animal feeding and medical implications [7]. In the zones of South Africa, Egypt, and South America, its fruit is also used for feeding the animals [8]. Similar types of the researches related to the *O. dillenii* proved that it may be used in the production of the nectar, sweeteners, jellies, jams and beverages of various types [9, 10]. *O. dillenii* is also grown in the sandy areas of Egypt as it has high drought resistance quality is widely used source of animal food and also act as air resistance during the storm [11]. *O. dillenii* has been used as a folk medicine to treat health disorders in several countries. This botanical is also used to prepare several cosmetic products like creams, lotions, and shampoos. In food industry it is being used to prepare as wines, jams [12]. Researchers have proved that many phytochemicals are present in different parts of cactus plant. *O. dillenii* have the potential against diverse environmental conditions [13]. The alcoholic extract of *O. dillenii* has antibacterial, anti-inflammatory, hypoglycemic activities. It has been used as anti-diabetic agent for the treatment of diabetes due to its hypoglycemic property [14]. The scientists are researching on the phytochemical as well as other pharmacological actions of this botanical in order to explore the use of this plant for prevention, management and treatment of many dreadful diseases [8]. Different plants were utilized for the synthesis of NPs including *Aloe vera* [15], *Cicer arietinum* [16], *Cymbopogon citratus* [3], and *Argemone mexicana* [17].

Plant crude extracts and their phytoconstituents with proven biocidal properties in therapeutic medicine are extremely important. In more recent years, various surveys have been conducted in various countries to demonstrate this efficacy. The secondary metabolites produced by the plants are thought to be responsible for the plants' antibacterial properties. As a result, these plants are commonly employed for therapeutic purposes. The antimicrobial ability of plant bioactive substances has been proven through phytochemical screening that vascular plants could be a source of unique antimicrobial properties [18]. Phytochemicals are preferable to manufactured biomolecules because they have no or low toxicity in humans [19]. This feature makes them ideal candidates for drug manufacture and development [20]. The discovery of novel antifungal medicines relies heavily on phytochemicals without endangering human health [21]. The present study is aimed at developing gold (Au) and silver (AgNPs) from the aqueous extract of *O. dillenii* and at exploring their antimicrobial potential.

2. Material and Methods

2.1. Collection of Plant. O. dillenii plant was collected from various regions of district Nowshera, Khyber Pakhtunkhwa, Pakistan. The plant was identified by professional taxonomist Dr. Muhammad Ilyas in the Department of Botany University of Swabi, Pakistan. The voucher specimen UOS-Bot-SP NO-101 was deposited in herbarium of Department of Botany University of Swabi, Pakistan. The plant material was dried under shade and grinded and crushed by grinder machine. The powder was soaked in organic solvent methanol for a week and then filtered. The filtrate was concentrated in rotatory evaporator. The crude methanolic extract was suspended in water and successively partitioned with *n*-hexane and chloroform, ethyl acetate and methanol.

2.2. Extract Preparation. The fine powder (1 kg) of the plant material was divided into three parts 550 gm, 350 gm, and 100 gm. About 550 gm of plant material were dipped in methanol, 350 gm in distilled water and 100 gm in *n*-hexane. The plant material was kept for one week in order to obtain crude extract. The obtain extract will be concentrated in rotatory evaporator to obtain crude *n*-hexane, methanolic and aqueous extract. Three different fractions were prepared in three different solvents to check the presence of most of the active ingredients, the active ingredients involved in capping and biological activities. As in *n*-hexane only nonpolar ingredients were determined and they are not involved in the capping during nanoparticle synthesis. The methanolic fraction will be applied next time for the synthesis of Ag and AuNPs, and biological activities. The plant material was stored in refrigerator for further NP synthesis.

2.3. Phytochemical Analysis. Phytochemical screening was performed to identify bioactive secondary metabolites. The glycosides, alkaloids, tannins, saponins, flavonoids, anthraquinones, betacyanins, phlobatannins, anthocyanins, emodins, steroids, carbohydrates, and terpenoids were screened using the specific tests.

2.4. Synthesis of NPs. 1 mM solution of silver salt ($AgNO_3$) and gold salt [$HAuCl_4$] were prepared for the synthesis of Ag and AuNPs by using plant crude extract at different ratio 1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5. Then, the solution was placed on stirrer with constant stirring at room temperature for 5 hours. After that, characterization of NPs was carried out.

2.5. Pharmacological Activities. The crude extract and synthesized NPs were evaluated for antibacterial and antifungal activities.

2.6. Chemical Used. The analytical grade chemicals were used in the synthesis of Au/AgNPs. $AgNO_3$ was purchased from Sigma-Aldrich, and $HAuCl_4$, methanol, and deionized water were purchased from Merck.

2.7. Biosynthesis of AgNPs Using the Aqueous Extract of O. dillenii. 100 ml of the already prepared extract was taken. 1 mM solution of $AgNO_3$ was prepared using distilled water. Different fractions of the preparations were prepared using Ag and extract solution in different ratios such as 1 : 1, 2 : 1,

3 : 1, 4 : 1, 5 : 1, 6 : 1, 7 : 1, and 8 : 1, respectively. The fraction was prepared in vials and kept on stirring for 24 hours. The change in color of the reaction mixture in each vial indicates the formation of NPs. The solutions were evaluated using a UV-visible spectrophotometer for the confirmation of AgNPs synthesis. The result of the UV-visible spectrophotometer revealed that the optimized ratio for AgNPs was 7 : 1.

2.8. Biosynthesis of AuNPs from the Aqueous Extract of O. dillenii. 1 mM $HAuCl_4$ was prepared using deionized water. Different ratio of Au salt solution and plant extract were taken such as 1 : 1, 3 : 1, 5 : 1, 6 : 1, 7 : 1, 8 : 1, and 9 : 1 in vials and kept for stirring for a period of 4 hours. The absorption spectrums were recorded for each fraction using a UV-visible spectrophotometer. The 8 : 1 was observed to be the optimized ratio of gold and extract for the preparation of AuNPs. Effects of other factors such as pH effect, NaCl effect, different salt effect, kinetic study, and temperature were also examined. The characterization of AuNPs was done using FTIR, SEM, EDX instruments.

2.9. Characterization of AgNPs and AuNPs

2.9.1. UV-Visible Spectroscopy. Initially the NPs were characterized by UV-visible spectrophotometer in the wavelength ranging from 200 to 800 nm. The spectroscopic analysis for both silver and gold NPs were carried out by using freshly prepared fractions at 37-38°C and by using optical path 1 cm length of quartz cuvettes using spectrometer (300 Plus Optima Japan). The AgNPs solution gave an absorption maximum at 420-450 nm while that of AuNPs was 520-530 nm.

2.9.2. Scanning Electron Microscopy (SEM). The size and morphological surface of NPs were determined using JEM 2100, Jeol CRL Scanning Electron Microscope (University of Peshawar, Pakistan).

The size and shape of the AgNPs and AuNPs were determined using SEM images. By using an electron microscope, a layer of AuNPs thin sediment was placed under vacuum pressure of 5-8 Torr.

2.9.3. Fourier Transform Infrared (FTIR) Spectroscopy Analysis. The functional group involved in the formation of Au and AgNPs was evaluated using Shimadzu FTIR - 8400-S (AWKUM) Fourier transform spectrometer. The samples were prepared using the powdered sample. The powdered samples were placed in NaCl cells and were placed in pellet cells of KBr. The bands detected on the computer showed the results. The range of 4000-400 cm^{-1} was used.

2.9.4. Energy-Dispersive X-Ray (EDX) Spectroscopy Analysis. Au and AgNPs geometry and morphology were also determined. Using a Bruker X-flash in energy dispersive X-ray spectroscopy the colloids of Au and AgNPs were prepared determined. For EDX and imaging 15 keV energy of the electron beam was maintained.

2.9.5. Antibacterial Activity. The MIC and MBC which are commonly known as minimum inhibitory concentration and Minimum bactericidal concentration, respectively, of AgNPs and AuNPs were also determined in the microbial activity.

The bacteria *S. aureus* and *E. coli* were used in the microbial assay. Micro dilutions of the NPs were prepared. The bacterial culture was prepared using nutrient agar and incubated for 24 hours at a temperature of 37°C. NPs dilutions were also employed at a specific area to evaluate the MIC of NPs.

The NPs containing Petri dishes were also incubated at the temperature of 37°C and for 24 hours. MBC or minimum bactericidal concentration evaluation of AuNPs and AgNPs was also determined. The lowest dose or dilutions of Au/AgNPs were used to test the area of inhibition. The process was repeated twice to get accurate results.

2.10. Antifungal Activity. The micro dilution plate assay method was used to determine fungicidal activity. The fungus species of *Candida albicans* was used in the assay. 20 mM buffer solution of sodium phosphate was mixed with 20 μl of both Au and AgNPs dilution 5, 2.5, 1.25 mg/ml in water. The sample was incubated at a temperature of 37°C for 2 hours.

The NP visibility loss was calculated using the following formula:

$$\left[1 - \left(\frac{\text{colony-forming unit in the presence of NPs}}{\text{CFUs with no particles}}\right)\right] \times 100]. \quad (1)$$

2.11. Stability of NPs. The stability of Au and AgNPs were checked against varying pH, different concentration of NaCl, same concentration of different salt and heat effect. After each treatment UV-visible spectra were recorded.

2.12. Kinetic Study of Synthesis of NPs. For the time-dependent synthesis of Au and AgNPs, samples were drawn from reaction mixture at regular interval of time and UV-visible spectra were recorded.

2.13. Phytochemical Analysis. The qualitative screening for the assessment of phytochemical components like flavonoids, terpenoids, alkaloids, carbohydrate and steroids, in the methanol, *n*-hexane, and distilled water extracts of the plant was carried out.

3. Results and Discussion

3.1. Phytochemical Screening. The qualitative screening for the assessment of phytochemical components like flavonoids, polyphenols, terpenoids, alkaloids, carbohydrates, and steroids in the methanol in each extract was performed by using the following reagents (Table 1).

The results of qualitative screening for the assessment of bioactive secondary metabolites such as flavonoids, polyphenols, terpenoids, alkaloids, carbohydrates, and steroids in the methanol, *n*-hexane, and distilled water extracts of the title plant are given in Table 2. The methanol plant extracts of *O. dillenii* showed presence of steroids, coumarins,

Table 1: List of reagents used for identification of secondary metabolites in extracts.

Phytochemicals	Method	Results for the presence of an ingredient
Alkaloids	0.5 g plant extract+2% H_2SO_4+Dragendorff reagent	Orange red ppt
Tannins	Filtered extract+drops of ferric chloride	Dark green color
Glycosides	Extract+HCl+NaOH+Fehling reagent	Appearance red ppt
Saponins	Extract+H_2O (boiled)	Frothing occur
Anthraquinones	0.5 g extract+10% HCl (boil) and then add chloroform+10% ammonia and heat	Appearance rose pink color
Flavonoids	0.2 g extract+NaOH+HCl	The yellowish color disappears on adding HCl
Steroids	0.3 g extract+acetic acid (heat)+H_2SO_4	Color change to green
Terpenoids	0.2 g extract+2 ml chloroform+3 ml H_2SO_4	Reddish brown color
Emodin	0.5 g extract+2 ml NH_4OH+3 ml benzene	Appearance of red color
Anthocyanin and betacyanin	0.2 g extract+2NaOH (heat)	Appearance of bluish green color

Table 2: Phytochemical evaluation of methanol, *n*-hexane, and distilled water plant extracts of *O. dillenii*.

Constituents	*Opuntia dillenii* plant extract		
	Methanol	*n*-Hexane	Distilled water
Steroids	+	−	−
Terpenoids	+	+	+
Glycosides	−	−	−
Flavonoids	+	−	+
Saponins	+	−	+
Carbohydrates	−	−	−
Tannins	+	−	−
Coumarins	+	−	−
Betacyanin	+	−	−
Anthraquinones	−	−	−
Anthocyanin	−	−	−
Emodins	−	−	−
Phlobatannins	−	−	−

"+" shows the presence and "−" shows the absence of the compound.

betacyanin, terpenoids, tannins, flavonoids while the *n*-hexane plant extract confirms the presence of saponins, terpenoids, flavonoids, and coumarin and the distilled water plant extracts of *O. dillenii* revealed saponins, terpenoids, flavonoids presence and the absence of carbohydrate, glycosides, anthraquinones, anthocyanin, emodins, and phlobatannins.

3.2. Characterization of Au and AgNPs

3.2.1. UV-Visible Spectroscopy. UV-visible spectroscopy is the technique which is considered as the colorimetry extension, it works on the principle of the absorption of light from the test sample, by using various components that are similar to the colorimeter but spectroscopy has the advantage of improved accuracy in a wide range of wavelengths between 190 and 700 nm [22].

This wavelength range was used because the absorption by the Au and Ag is usually observed in this range. In this work, we used a 300 Plus Optima Japan Spectrophotometer, with quartz cells and 1 ml deionized water as a blank. The formation of the NPs was confirmed by the formation of color visually. Scanning the absorption using wavelength ranges of 200 and 800 nm was used to confirm the formation of the Au and AgNPs. Spectra show the band wavelength of the Au and AgNPs obtained using the plant extract. The difference in the bands and the wavelengths indicate the different sizes and the shapes of the Au and AgNPs obtained (Figure 1).

The prominent UV-visible spectrum was observed at the wavelength of 543 nm and 445 nm which confirmed the synthesis of Ag and AuNPs, respectively (Figure 1). The UV-visible absorption spectrum of the aqueous extract *O. dillenii* has not shown any significant bands. But after the extract of *O. dillenii* with the silver nitrate colorless and chloroauric acid yellow colored solution the color of the solutions were changed to characteristic ruby-red color and reddish-brown showing the convenient excitation due to the surface Plasmon resonance phenomenon, that indicates the silver and gold NPs formation [23]. This research also observed the synthesis of Au and AgNPs within 15 to 20 minutes in the case of silver and 10 to15 minutes in the case of the synthesis of gold NPs, respectively.

3.2.2. Scanning Electron Microscopy (SEM). The analysis of the size and shape of the Au and AgNPs was determined using SEM. For this purpose, SEM images and photographs of SEM using JEM 2100, Jeol CRL, Scanning Electron Microscope. By using an electron microscope, a layer of Au and AgNPs thin sediment was placed under vacuum pressure of 5-8 Torr.

The results of the SEM analysis revealed the shape of the NPs were roughly spherical-shaped NPs and in some areas stacked together. The characterization results of the Au and AgNPs by SEM analysis also confirm the method developed was suitable and effective to obtain the silver and AuNPs of different sizes and in diverse shapes (Figure 2). The size distribution of the NPs ranges from 45 nm to 77 nm approximately and was uniformly distributed.

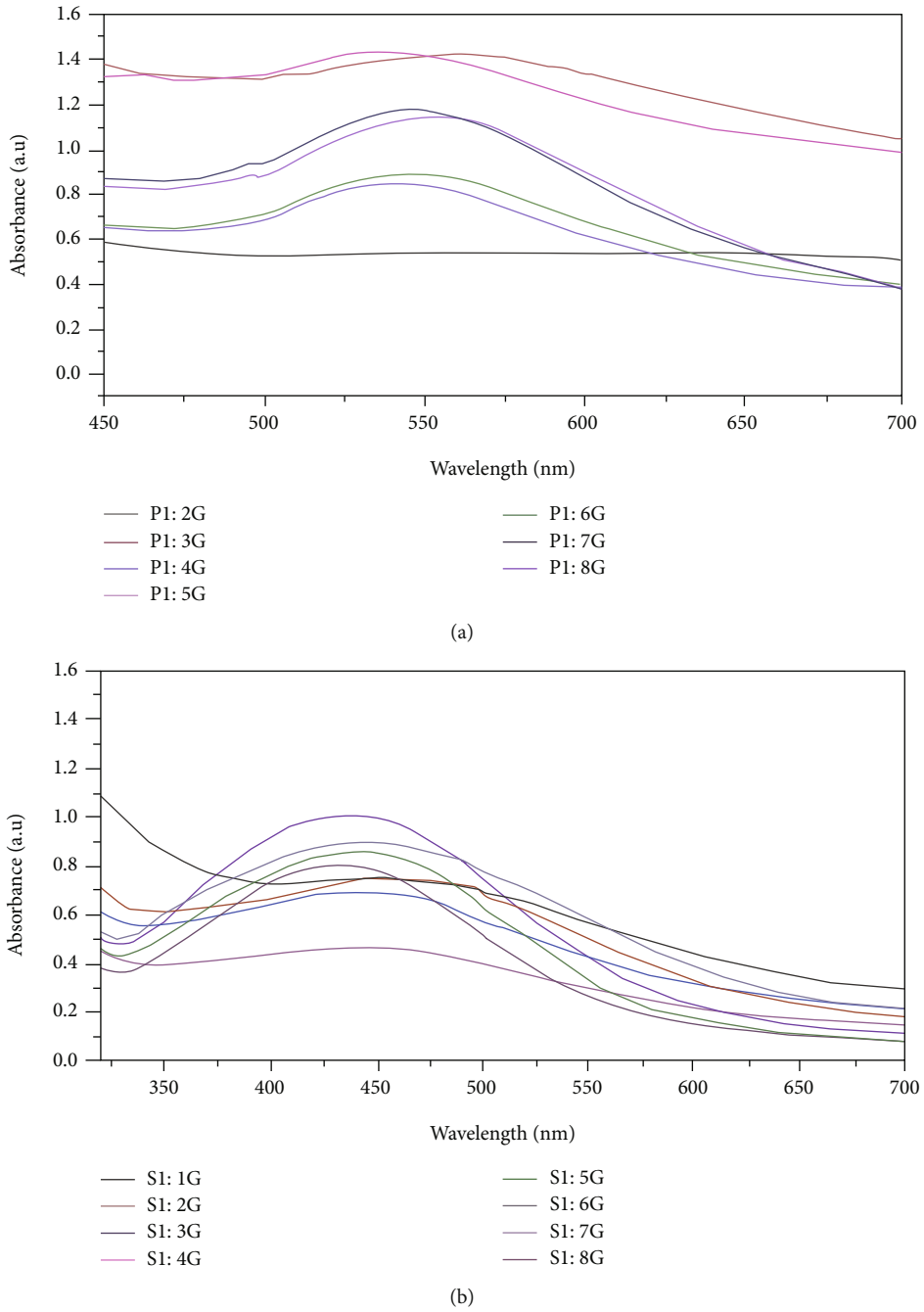
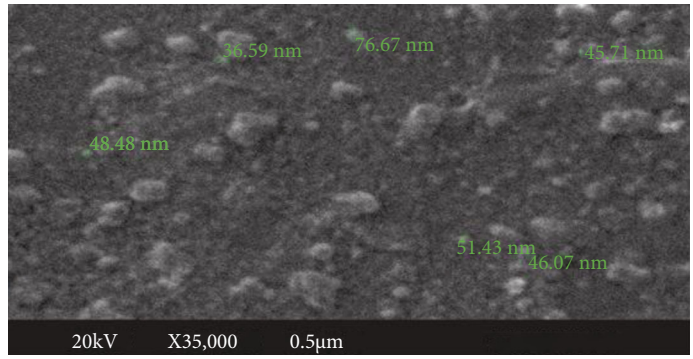


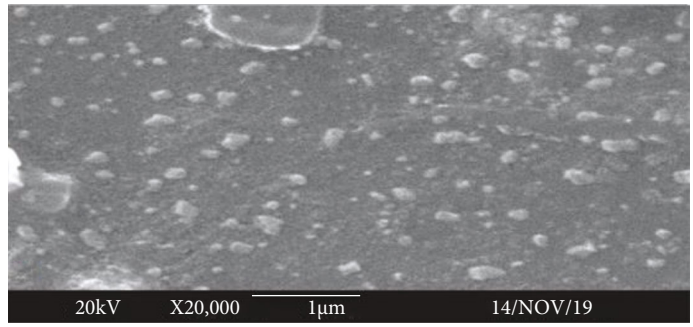
Figure 1: UV-visible spectrum of the synthesized AuNPs (a) and AgNPs (b).

3.2.3. Fourier Transform Infrared (FTIR) Spectral Analysis. The detection of functional groups present on Au and AgNPs was evaluated using Shimadzu FTIR - 8400-S (AWKUM) Fourier transform spectrometer. The range of 4000-400 cm^{-1} was used. The spectrum obtained by the analysis of the Au and AgNPs are given in Figure 3. The spectrum of FTIR of the extract containing *O. dillenii* obtained by employing the colloidal solution of silver and gold NPs envisages the different molecular arrangements of various functional groups present as shown in Figures 3, respectively. When different obtained transmission bands were compared, in the colloidal solutions, it resulted in the increased/suppressed bands that were because of the consequence of metallic NPs which were bound to the bioorganic molecules. The shifted bands were the characteristics of C=O and O–H modes of stretching which occur because of the free carboxylic acid, hydroxyl

group, respectively. Another shift in the band could be due to the vibrations of the C–OH group of the primary alcohol present in the extract solutions. FTIR spectra obtained provide clear evidence of the presence of various functional groups in the extract of *O. dillenii* which can also



(a)



(b)

Figure 2: SEM analysis of AgNPs (a) and AuNPs (b).

act as the capping or reducing agents of the Au and AgNPs. Correspondingly the results of these studies were observed compared to the study in which a similar phenomenon was observed to evaluate the biological molecules acting as the capping and reduction agents for Au and AgNPs [24].

3.2.4. Energy-Dispersive X-Ray (EDX) Spectroscopy Analysis. Au and AgNPs geometry and morphology were also determined. Using a Bruker X-flash in energy dispersive X-ray spectroscopy the colloids of Au and AgNPs were prepared. For EDX and imaging 15 keV energy of the electron beam was maintained. The obtained spectra are given in Figure 4.

The synthesized Au and AgNPs were further characterized qualitatively as well as quantitatively using EDX analysis, which revealed the highest signal proportion of Au and Ag in the solutions as shown in Figure 4.

The results of the EDX analysis confirmed the major metal in the precipitate were Au and Ag, respectively. The results obtained were consistent with the outcome of the EDX analysis. The identification of Cl in the spectrum was due to the ions of salt in the sample. The energy bands for a strong signal of Au were in the range of 2 to 2.5 keV, 9.5 to 10 keV, and 10 to 11 keV and for Ag, it was observed in the range of 2.9 to 3.8 keV which were similar to the observations and evaluations of the study of Au and AgNPs [25, 26].

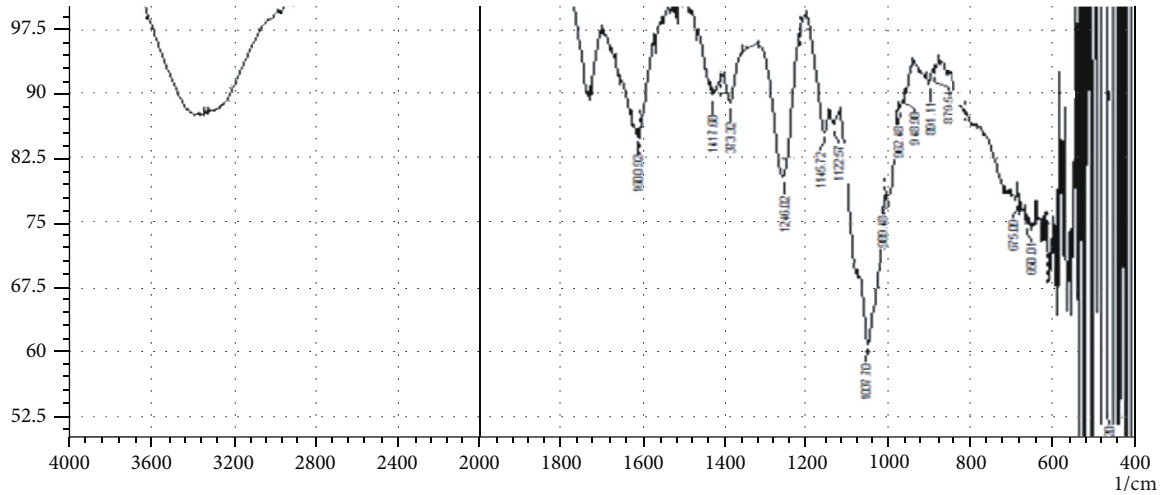
The EDX analysis of these NPs also shows the presence of other elements in the precipitate mainly carbon, aluminum, sulfur. Oxygen was also identified in the spectrum of the precipitate, which is assumed to be associated with the metals as the oxides or hydroxide of metals. For the presence of grid composition, Si showed the band. Minor carbon bands were also observed that may be due to the biomolecules which were bound to the NPs surface.

The bands of C and O along with the bands of metal signals suggested the Au and AgNPs may be capped due to the presence of phytochemicals of the plant extract by the atoms of an oxygen atom or may be related due to the existence of consequent oxides of metals which were identified in sample precipitate [25].

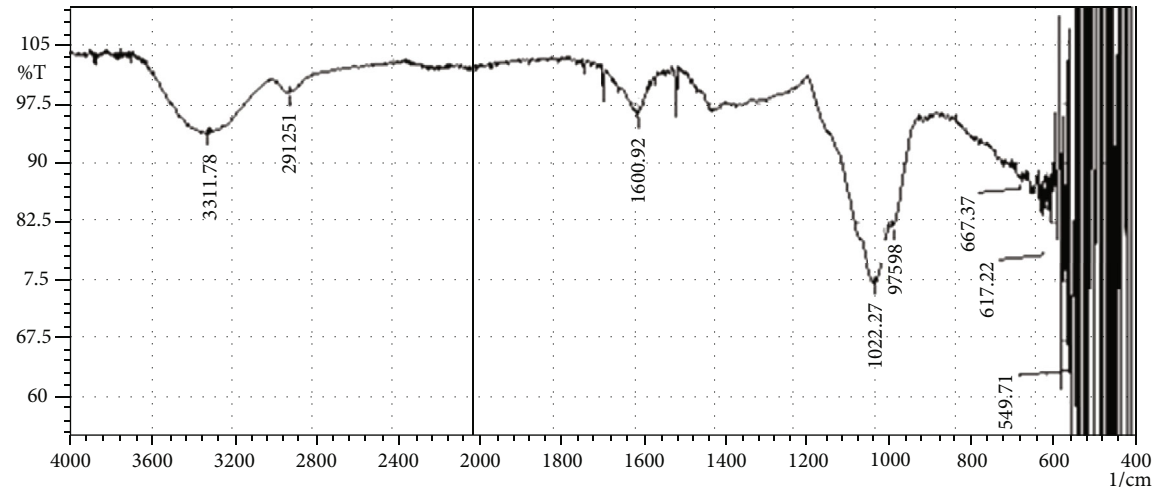
3.3. Antibacterial Activity. The MIC and MBC which are commonly known as minimum inhibitory concentration and Minimum bactericidal concentration, respectively, of Au and AgNPs were also determined in the microbial assay using *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, and *B. subtilis*. These results are obtained by comparing with standard.

The antibacterial activity of the extract of *O. dillenii* was in range of 11 to 14 mm against selected bacterial strains *S. typhi*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli* while for AgNPs, the zone of inhibition was 11 to 16 mm and for AuNPs, it was 11 to 17 mm. The Au and AgNPs exhibited good antibacterial property against various strains of bacteria as shown in Table 3.

3.4. Antifungal Activity. The microdilution plate assay method was used to determine fungicidal activity. The fungus species of *C. albicans*, *A. niger*, and *P. notatum* were used in the assay. In this assay of spot plating, the antifungal activity of the *O. dillenii* extract, and its synthesized Au



(a)



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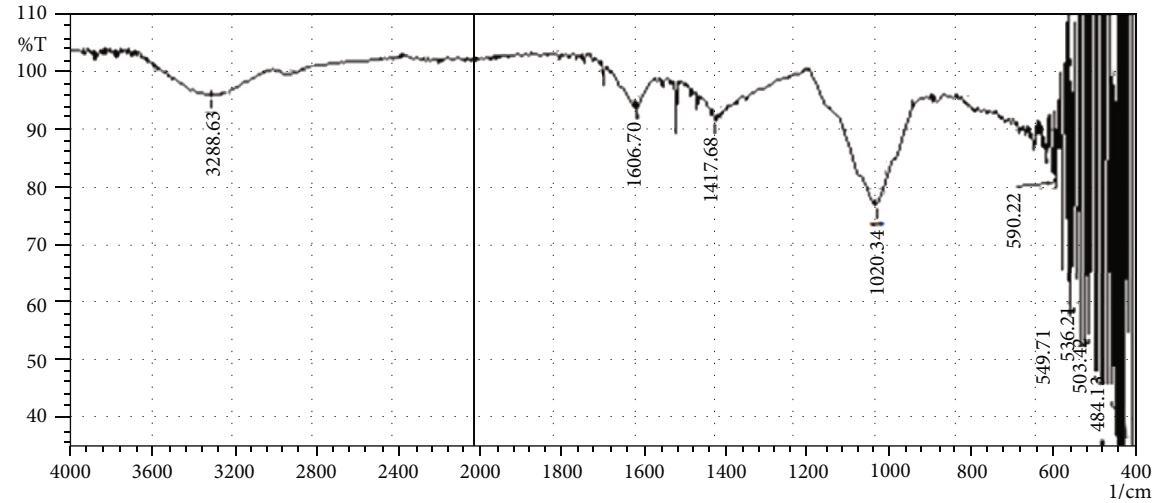
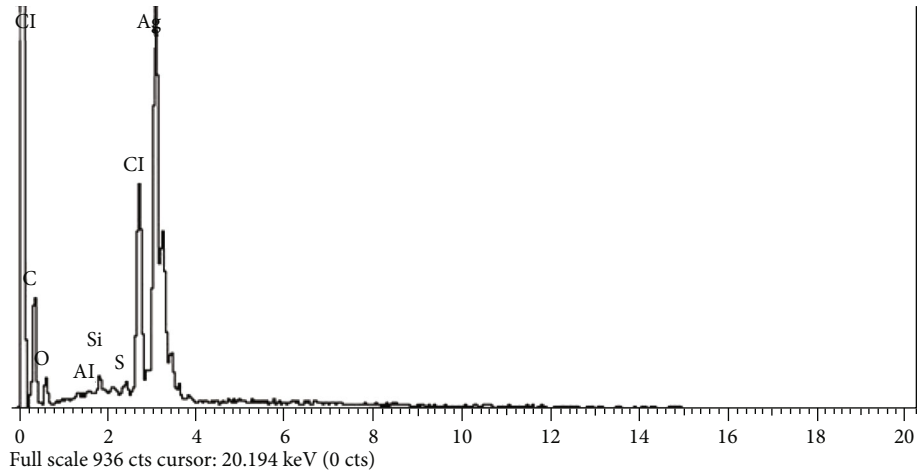
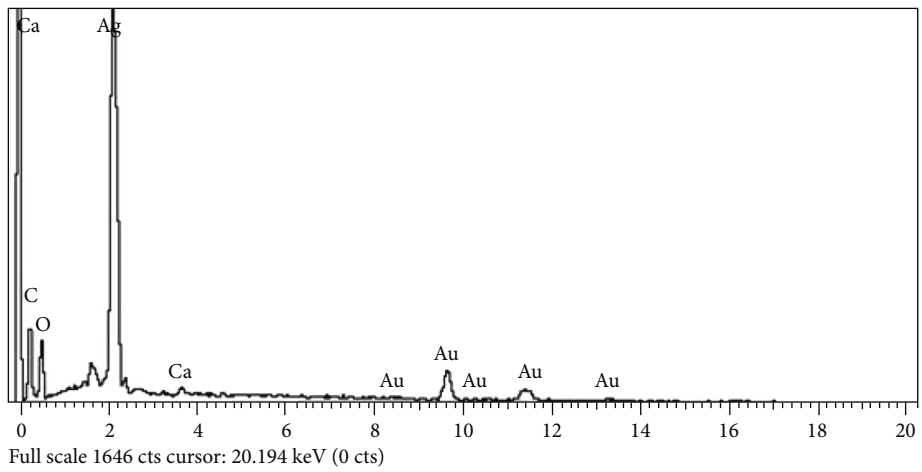


(c)

Figure 3: FTIR spectra of (a) extract, (b) AuNPs, and (c) AgNPs.



(a)



(b)

Figure 4: EDX spectrum of AgNPs (a) and AuNPs (b).

Table 3: Antibacterial activity of extract, AgNPs, and AuNPs of *O. dillenii*.

S. no	Samples	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*
1	Plant extract of *O. dillenii*	14.24 ± 1.67	13.32 ± 1.54	11.09 ± 1.11	12.65 ± 1.23	14.34 ± 1.08
2	AgNPs of *O. dillenii*	16.11 ± 1.65	15.43 ± 1.43	11.23 ± 1.20	12.12 ± 1.98	15.43 ± 1.00
3	AuNPs of *O. dillenii*	16.34 ± 1.98	17.20 ± 1.43	13.21 ± 1.65	13.54 ± 1.88	13.33 ± 1.23
4	Amoxicillin (standard)	23.65 ± 1.54	26.98 ± 1.32	25.77 ± 1.43	29.09 ± 1.98	29.66 ± 1.43

Table 4: Comparative antifungal activity of the aqueous extract, AgNPs, AuNPs, and standard antifungal drugs.

S. no	Samples	*Candida albicans*	*Aspergillus niger* Zone of inhibition (mm)	*Penicillium notatum*
1	Extract of *O. dillenii*	12.76 ± 1.23	11.76 ± 1.65	13.23 ± 1.54
2	AuNPs of *O. dillenii*	22.03 ± 1.00	19.98 ± 1.43	21.65 ± 1.09
3	AgNPs of *O. dillenii*	24.23 ± 1.29	21.54 ± 1.09	27.76 ± 1.65
4	Miconazole (standard drug)	100.00 ± 0.02	100.00 ± 0.08	100.00 ± 0.04

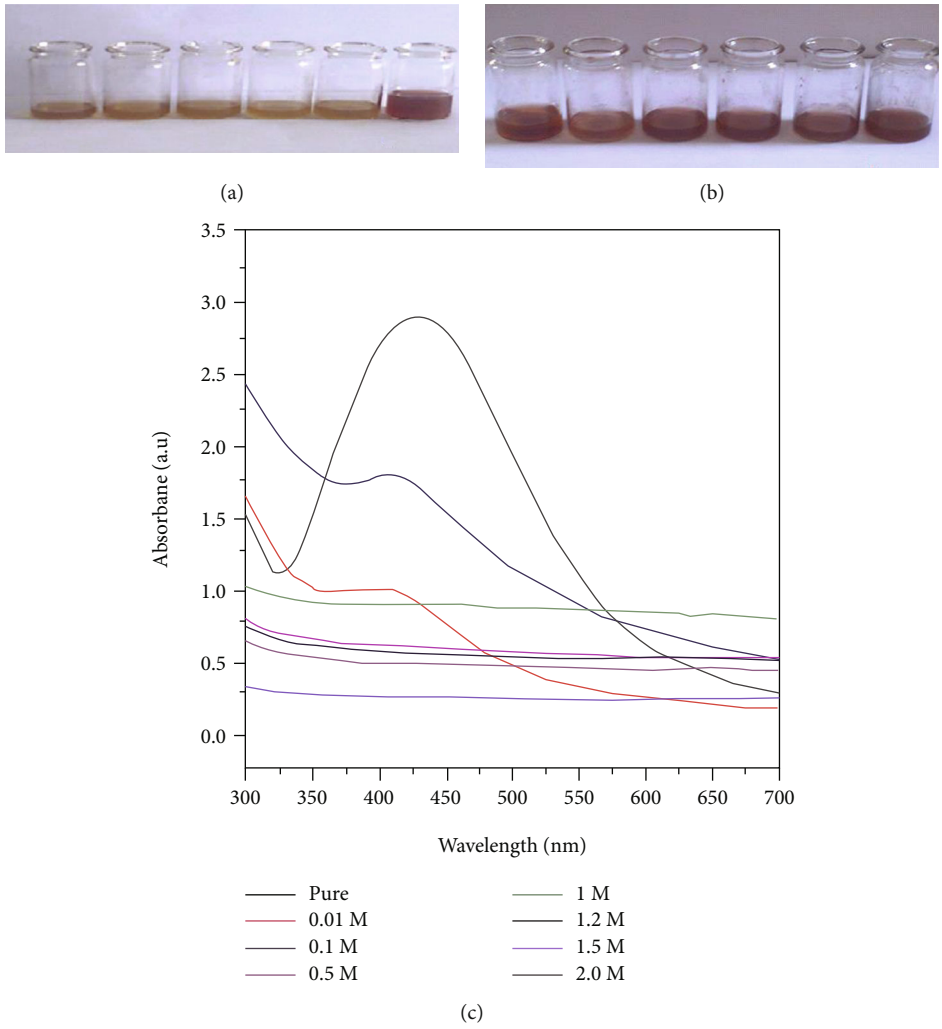


Figure 5: Continued.

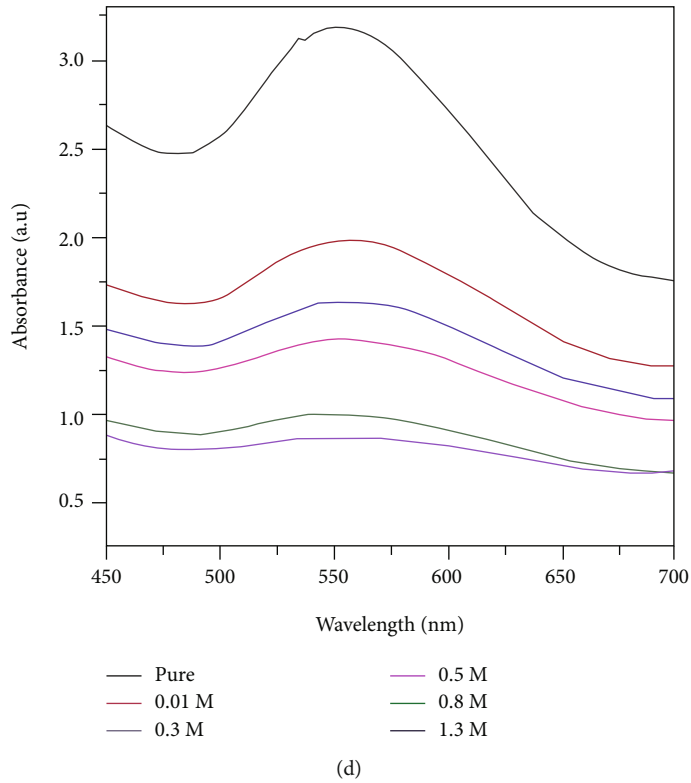


Figure 5: Visual effect of NaCl on of AgNPs (a) and AuNPs (b). UV-visible data of NaCl effect on AgNPs (c) and AuNPs (d) of *O. dillenii.*

and AgNPs was tested in a growing medium. This assay ascertained whether the division and the growth are prerequisites for Au and AgNP antifungal activity. The results of the antifungal activity are given in Table 4.

These results of the antifungal assay confirmed that extract of *O. dillenii* contain only mild antifungal activity while the Au and AgNPs possess some antifungal activity. The antifungal activity exhibited by extract of *O. dillenii* against different fungal strains ranged between 11 and 13 mm. The antifungal activity 13 mm was found against *P. notatum*, followed by *C.albicans* (12 mm) > *A.niger* (11 mm). The antifungal activity of AuNPs of *O. dillenii* against different fungal strains ranged between 19 and 22 mm. The antifungal activity 22 mm was noted against *C. albicans* followed by *P.notatum* (21 mm) > *A.niger* (19 mm). Likewise, the antifungal activity of AgNPs of *O. dillenii* against different fungal strains ranged between 21 and 27 mm. The antifungal activity 27 mm was found against *P. notatum* followed by *C.albicans* (24 mm) > *A.niger* (21 mm) as shown in Table 4.

3.5. Stability Factors

3.5.1. Effect of NaCl on Ag and AuNPs. Effect of different concentrations of the NaCl was performed on the solutions of Au and AgNPs using pure, 0.01, 0.1, 0.5, 1, 1.2, 1.5, and 2 M solution of NaCl for AgNPs and 0.1, 0.3, 0.5,0.8, 1.3 and 1.5 M NaCl solution for evaluating stability on AuNPs. The solutions were made by dissolving the required amount of NaCl in specific volume of distilled water. 0.5 ml of all the prepared molar solution was added in 2 ml of Au and AgNP solution and was shaken well, and the effect of different NaCl concentrations on the NPs was evaluated using a UV-visible spectrophotometer and spectrum was drawn based on the absorbance values on different wavelengths ranging from 200 to 800 nm as shown in Figure 5.

The Au and AgNP solution contains Au and Ag atoms, respectively, along with negatively charged chloride ions which allow the Au and Ag atoms to be dispersed in the solution. The NPs then starts forming agglomerate and change the solution color because the NPs absorb wavelength e.g., green, orange, red, and yellow excluding shorter wavelengths of purple and blue. Then, the color of the colloids changes to bluish by absorbing shorter wavelengths: green and blue and green instead of red and orange and red. By increasing the concentration of NaCl, the solution became colorless because of the no suspended particles in the solution they started forming precipitates and aggregates at the bottom therefore no absorbance of light occurred by the solution.

3.5.2. Effects of Different Salts on Au and AgNPs. The effects of the different ionic strengths on the size of both Au and AgNPs were also evaluated. The investigation of the NPs in different salts range of ionic strength is also necessary for assessment of the NPs behavior in the different salt environment since the relevant environmental conditions may vary according to different salts and their ionic strength.

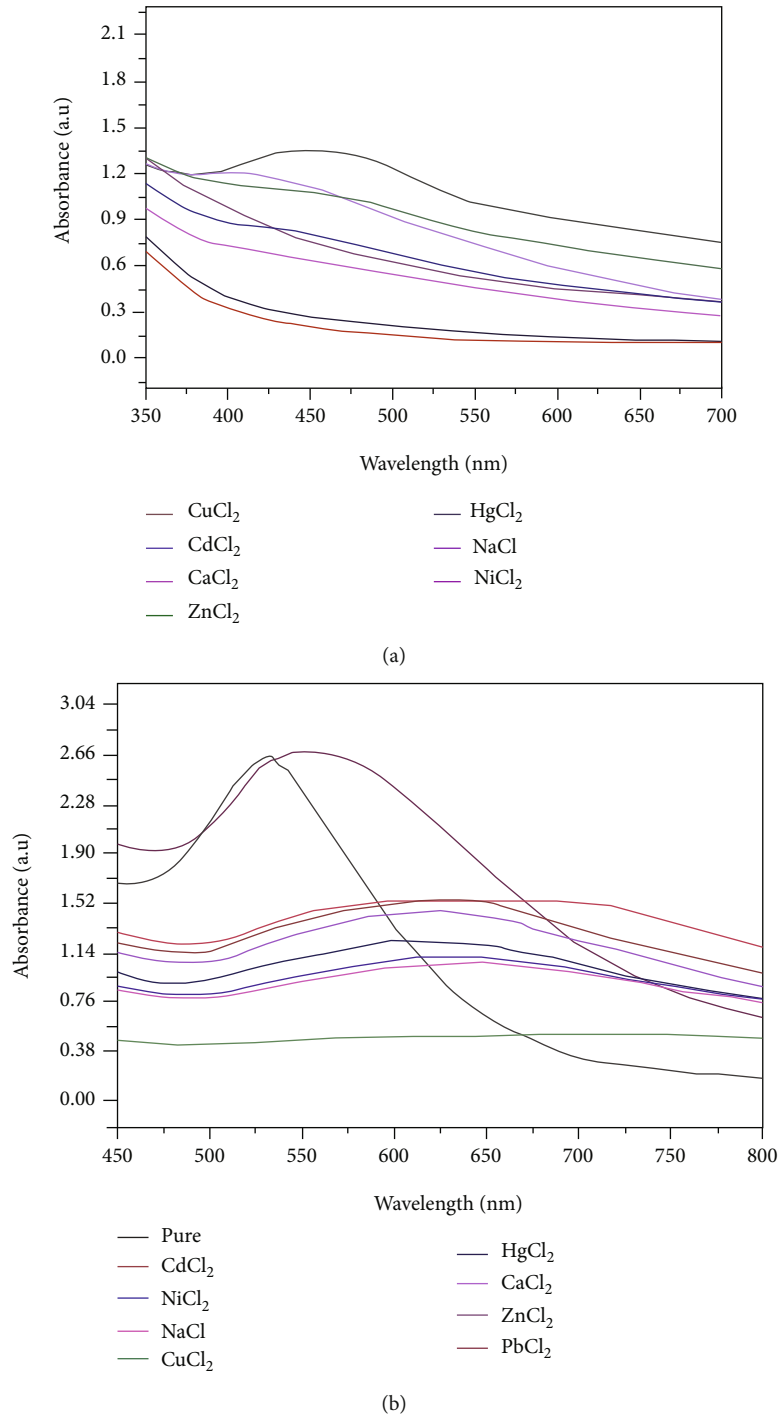
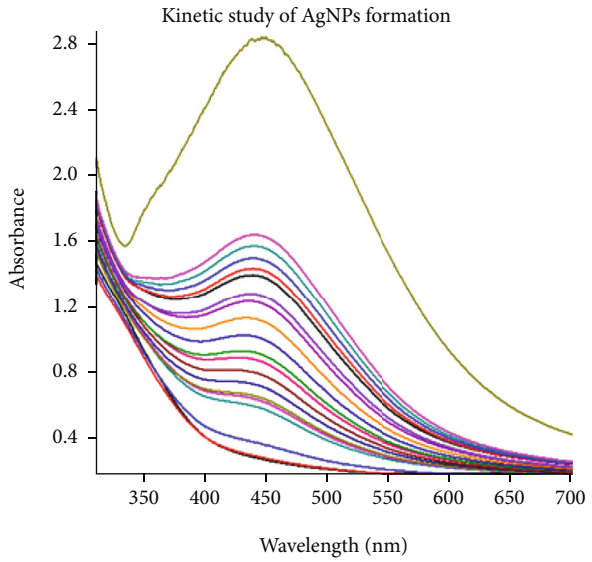


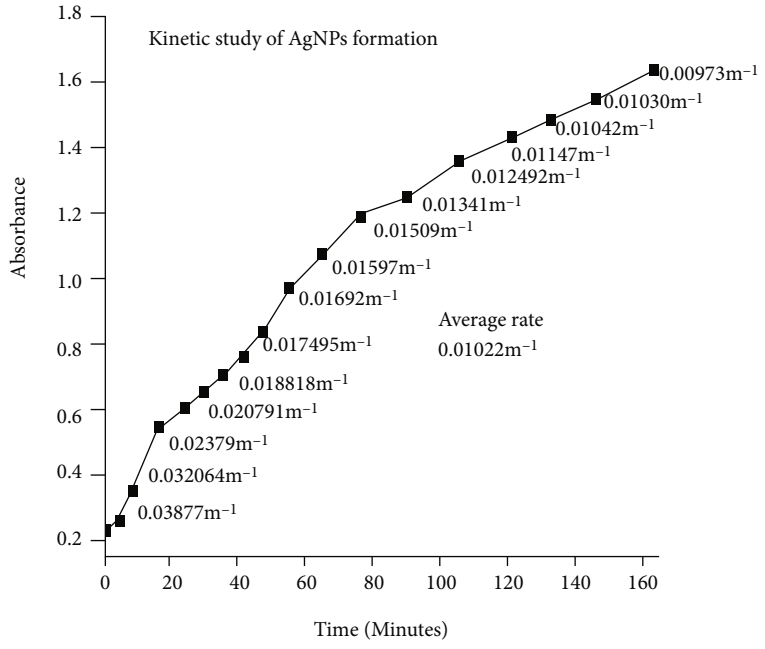
Figure 6: UV-visible spectra for effect of different salts on AgNPs (a) and AuNPs (b).

The salts used in the evaluation of the effects on Au and AgNPs were $NiCl_2$, $CuCl_2$, $HgCl_2$, $CaCl_2$, $ZnCl_2$, and $PbCl_2$.

The difference in the particle sizes as a function of the ionic strength is shown in Figure 6. As revealed from the spectrum steep gradient it is manifested that the AuNPs are highly susceptible to high salts strength as compared to the AgNPs. As the size of NPs increase it lead to formation of the aggregates in the media. The steepness in the spectrum shows the particle aggregation caused by ionic strength. The research by NPs evaluated and compared the effects of different electrolytes and concluded that salts with the divalent cations have a great influence on NPs aggregation in comparison to the salts with monovalent cations. The chloride ions also enhanced the NPs aggregation [27]. Likewise, Badawy also analyzed AgNPs and the consequence of different monovalent and divalent cations [28]. The Au



(a)



(b)

Figure 7: Continued.

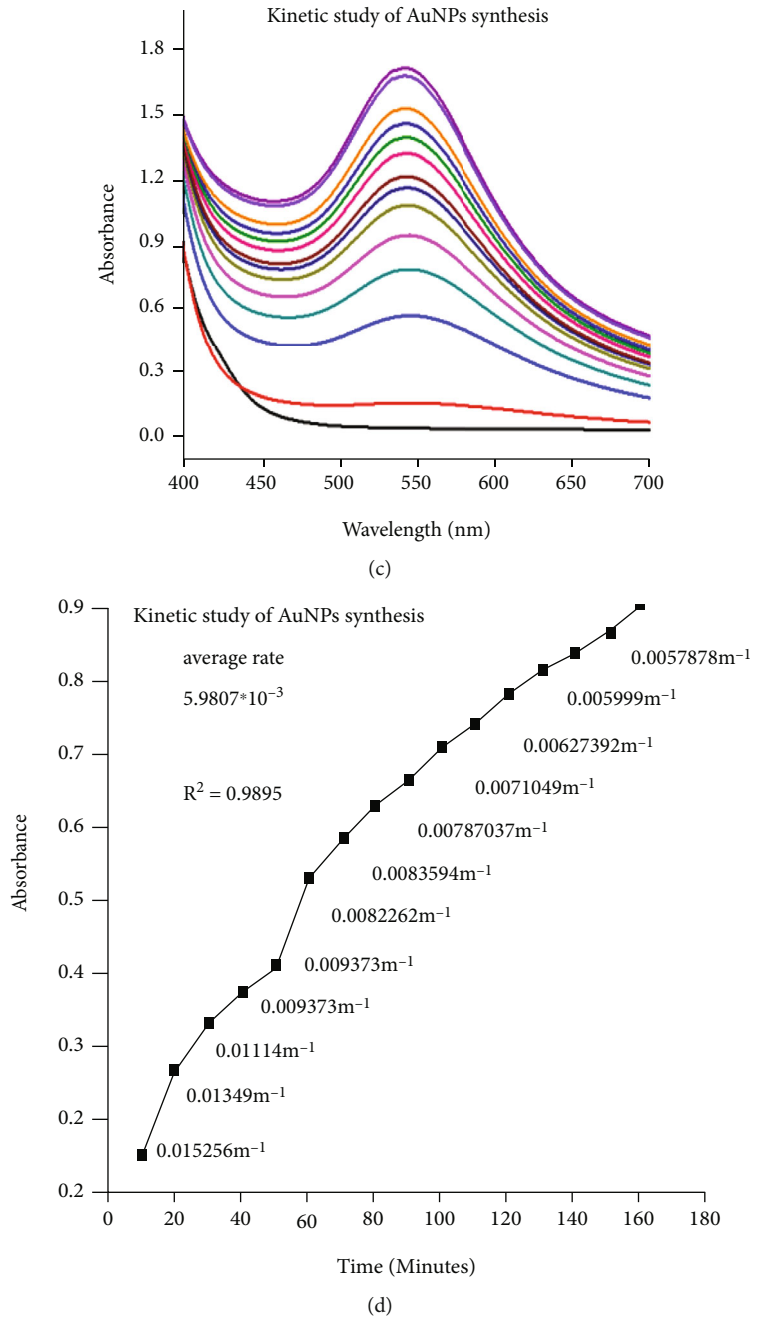


Figure 7: UV-visible data of kinetic study of AgNPs (a). Rate of reaction for the synthesis of AgNPs (b). UV-visible data of kinetic study of AuNPs (c). Rate of reaction for the synthesis of AuNPs (d).

and AgNPs, at a higher strength of ionic salts, have aggregated and Ca^{2+} cations presence have resulted in the enhancement of the apparent aggregation. The aggregation of the NPs has revealed that NPs were efficiently destabilized by divalent electrolytes [29].

3.5.3. Kinetic Studies of Au and AgNPs. The kinetic study of Au and AgNPs showed that with the passage of time the number and uniformity of NPs increases. When the absorbance was plotted against time, it gave almost a straight line, from which the rate of reaction was calculated (Figure 7). After specific intervals of time revealed that the production and the uniformity of the NPs increase with the increases in time, the slope bands in the spectrum showed the maximum production of the NPs in the solution.

3.5.4. Effects of Heat on Ag and AuNPs. The effect of the temperature on the NPs is considered as the major factor which can affect the synthesis of Au and AgNPs. This analysis was conducted on different temperatures visually

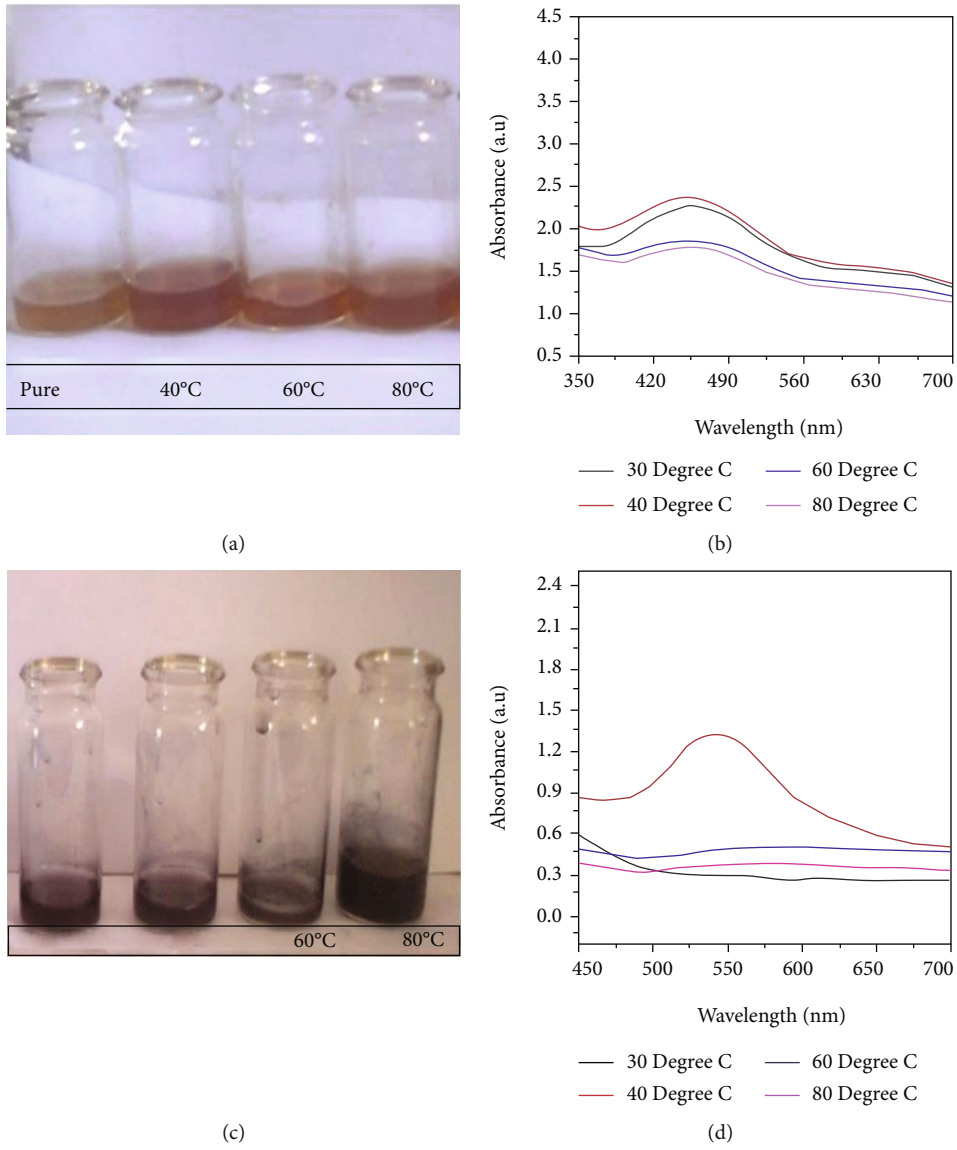


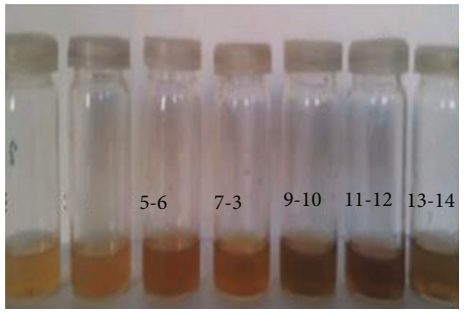
Figure 8: Visual effect of heat on AgNPs (a) and AuNPs (c) and UV-visible data of heat effect on AgNPs (b) and AuNPs (d).

shown in Figure 8 and was confirmed by analysis by the UV-visible spectra at three different temperatures 40°C, 60°C, and 80°C.

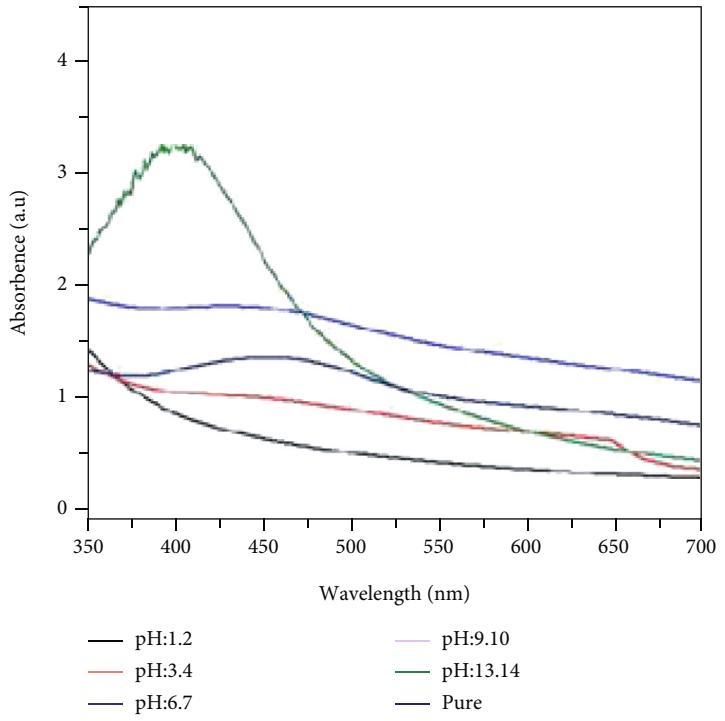
It can be observed clearly from the spectra of UV-visible spectrophotometer that synthesis of Ag and AuNPs increases with temperature but after certain temperature, it became destabilized which caused the NPs to clump together and agglomerated. It also caused the broadening of peak which indicate the big NPs that settled down. in the formation of Au and AgNPs with an increase in the temperature [30].

3.5.5. Effects of pH on Au and AgNPs. The effect on the Au and AgNPs was also studied due to the change in the pH was studied in different conditions including the pH ranges from 1 to 14. The pH was adjusted by dropwise addition of HCl and NaOH. Figure 9 shows the visual analysis of pH on NPs, and the effect of changes in pH on UV-Vis spectra of Au and AgNPs synthesized is shown in Figure 9.

The effect of pH on stability of NPs can be observed by change in the color of NPs solution [31]. At low pH ranges small the broadening of the bands was formed which indicates the formation of large-sized NPs. In the extract mediated synthesis of NPs alkaline pH shows the narrowing of the band at 400 nm with maximum sharp band production. The formation of the sharp band indicates the formation of the spherical shape of NPs [32]. Several studies reported that pH plays a vital role in the determination of the size and shape control synthesis process of NPs. This research indicates that alkaline pH 6-7 is more suitable for the synthesis of Ag and 8-9 for AuNPs. It was reported that AuNPs at pH 10 showed maximum stability using the extract of *M. charantia* [33].



(a)



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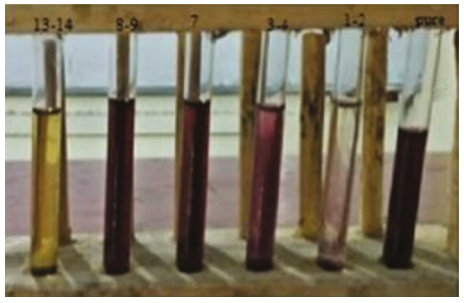
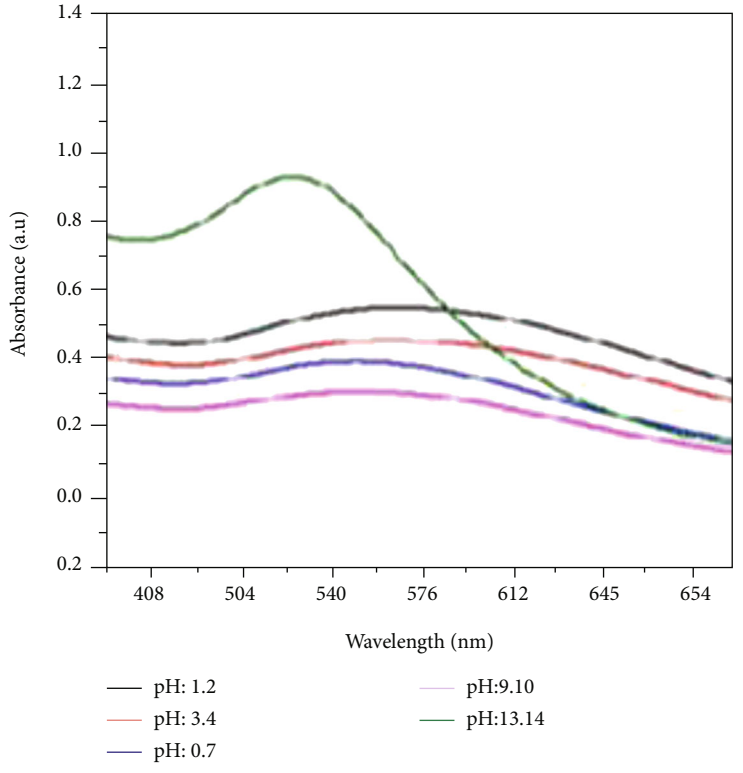


(c)



(d)

Figure 9: Visual effect of pH on AgNPs (a) and AuNPs (c) and UV-visible data of pH effect on AgNPs (b) and AuNPs (d).

4. Conclusions

The Au and AgNPs were characterized using UV, FTIR, SEM, and EDX spectra and showed the rapid biosynthesis of NPs using *O. dillenii*. An increase in the awareness of utilizing green chemistry and adopting the green route for the production of metallic nanoparticles leads to the development of efficient and ecofriendly techniques. The advantages

of the synthesis of Au and AgNPs by the use of plant extracts are being most economical, cost-effective, and energy-efficient and provide an efficient application towards the communities, protecting environment and health leading towards the lessening in the production of hazardous wastes and development of safe products. Green-synthesized Au and AgNPs have various significant nanotechnology aspects with matchless applications.

Data Availability

The data produced in this finding has been included in the main text of this paper.

Conflicts of Interest

The authors declare no potential conflict of interest.

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