Grewia asiatica leaves extract assisted green synthesis of gold nanoparticles and study of their antibacterial, antioxidant and photocatalytic potential

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Abstract

The environmental issues generated by industrial advancement and human activities are serious and must not be overlooked. Bio-nanotechnology has emerged as a solution for producing biosynthetic nanomaterials to address environmental issues. The current work describes for the first time an economical and environmentally benign method of producing gold nanoparticles utilizing *Grewia asiatica* (GAAuNPs). The optimal AuNPs were produced using a gold ion concentration of 1 mM and a metal-to-extract ratio of 1:1. The resulting nanoparticles were analyzed and characterized using X-ray crystallography, FTIR, SEM, EDX, and UV visible spectroscopy. The catalytic methylene blue (MB) dye degradation properties, antibacterial and biofilm inhibition abilities, and antioxidant properties of the synthesized particles were investigated. The Au NPs demonstrated efficient catalytic degradation against MB and completely degraded it in 8 minutes. The NPs also exhibited potent biofilm inhibition against *E. coli* and *S. aureus* that was close to the standard and was more antioxidant than the standard. The findings highlight *G. asiatica* as a suitable, inexpensive biosource for biofabricating GAAuNPs with extensive multifunctional uses. To conclude, GAAuNPs' ability to degrade organic pollutants and eradicate pathogens provides a cost-effective and environmentally benign remedy to tackle contemporary pollutants.

1. Introduction

Due to the global threat of resistant bacteria, food safety, economic growth, and human and animal health are all at risk (Man et al. 2019; Puvača et al. 2021). Additionally, bacteria significantly contribute to persistent and recurring infections because of their propensity to create biofilms, which shield them from the host's immune system, antibiotics, and disinfectants (Singh et al. 2018; Nassima et al. 2019). In today's world, it is essential to have an efficient and advanced plan for managing and treating drug-resistant microorganisms (Swolana et al. 2020). Similarly, an unfavorable ecological shift is accompanied by untreated industrial and domestic effluents being discharged into the ecosystem(Natasha et al. 2020). The waste dye molecules significantly negatively impact humans, wildlife, and vegetation. As a result, organic dye effluents are now recognized as a significant threat to aquatic environments (Iqbal et al. 2020; Ahmad et al. 2022). Organic dyes are the most hazardous of all the contaminants released into our environment. The primary contaminants in industrial effluent are dyes because of their color (Javed et al. 2021; Sher et al. 2021a). The biological breakdown of dye molecules is exceedingly difficult because they are tenacious compounds. Due to their toxicity, commercial significance, and environmental effects, dyes have been the subject of extensive research (Qamar et al. 2022b). As a result, pathogens and organic contaminants must be removed and detoxified from water bodies more urgently than other pollutants. Different remediation techniques, including chemical, physiochemical, and biological treatments, have so far been suggested. However, each has its pros and cons compared to the others(Sher et al. 2021a; Javed et al. 2023).

These colored pollutants are difficult to remove using traditional methods (such as adsorption, coagulation, flocculation, biodegradation, and so on), and all of these procedures are expensive and require additional planning to remove the byproducts. Alternatively, photocatalytic degradation of hazardous dyes into harmless chemicals is the preferred method for reducing their environmental impact. Developing an effective and long-term solution for disposing of harmful dyes and pathogens in wastewater streams is paramount. Recent advances in nanotechnology have enabled the use of nanoparticles as biological agents for suppressing bacteria and as catalysts for degrading toxic pollutants. Materials at the nanoscale are the focus of nanoscience, which has applications in many different areas, including medicine, engineering, forensics, agriculture, cosmetics, and even orthodontics (Cullen et al. 2020). Its broad application in applied sciences, one of the fastest-growing fields of scientific research, and technological advancements pique the interest of researchers (Irshad et al. 2021; Sher et al. 2021a; Chakravarty et al. 2022).

Nanoparticles made of metals such as silver, gold, platinum, nickel, manganese, titanium, and zinc exhibit non-toxicity, antibacterial activity, and catalytic activity. The unique surface plasmon resonance (SPR) properties of gold nanoparticles (AuNPs) make them highly suitable for use in biological fields. They are also easy to produce, can be adjusted in size, and are multifunctional. Furthermore, their properties are well-established. There are many different approaches to synthesis, including chemical and physical techniques; however, it is unfortunate that they all negatively affect the surrounding environment(Lee et al. 2020; Hashmi et al. 2021). As a result, there is a pressing need to produce metal oxide NPs that are harmless to the environment through simple techniques and plant extracts and diverse biological species(Rasheed and Meera 2016). In vitro, the green synthesis of NPs is becoming increasingly popular due to its low environmental impact, low production costs, and high efficiency(Soni et al. 2018). This method is easy, rapid, and only requires one step and is suitable for production on a commercial scale. This technique involves converting metal salts into particles and stabilizing them with the use of a biological templates. Metal and metal oxide NPs offer good chemical, physical, and biological capabilities against bacteria, fungus, and viruses due to their high surface area, composition, and shape (Qamar et al. 2021; Singh et al. 2021).

There are numerous methods for producing metal nanoparticles, including chemical and biological processes involving microorganisms and plants. (Singh 2022). Due to their extensive surface area, gold nanoparticles (Au NPs) have unique electrical, magnetic, and catalytic properties and vast biological applications (Chen et al. 2022; Carrapiço et al. 2023; Mitri et al. 2023). Au NPs are adaptable and resourceful and can be modified into various shapes depending on the need to switch from one task to another (Manjubaashini and Thangadurai 2023). It becomes a strong competitor for silver. These NPs can effectively attack all types of bacteria and viruses compared to silver and other metals and are less hazardous and toxic to the environment when compared to other chemically synthesized nanoparticles (Timoszyk and Grochowalska 2022). Because of their resistance to surface oxidation, stability, flexible surface characteristics, and low cytotoxicity, Au NPs are excellent for nanotechnologies such as drug delivery (Patel et al. 2022). *Grewia asiatica* known as phalsa in lower Punjab (Pakistan) is rich in essential nutrients. In the current study Au NPs were produced using *Grewia asiatica* (locally called phalsa) leaf extract. There have been no studies regarding gold nanoparticle synthesized from this locally occurring phalsa. It is used for instant cooling the body in summer, maintaining electrolyte balance and soothing joint aches, and effectively managing seasonal and chronic conditions(Swain et al. 2023). It has a high level of vitamin C, minerals, proteins, phenolics, flavonoids, tannins and anthocyanins. It seeds fruit pulp highly rich in phytochemicals is used to treat different diseases and effective in improving respiratory and cardiac functioning. Their fruit has anticancer, antioxidant, and anti-hyperglycemic characteristics, while the stem bark has analgesic and anti-inflammatory effects(Ranjan et al. 2022). Their leaves have antibacterial, anticancer, and antiemetic properties. Hence, the current work is highly motivated to synthesize gold nanoparticles of the smallest possible size using leaf extract of *Grewia asiatica* (GALE) and to explore the photocatalytic dye degradation, antioxidant and biofilm inhibition properties of synthesized nanoparticles (GAAuNPs).

2. Materials and Methodologies

2.1 Chemicals, Reagents and Plant Source

Chemicals utilized in the study were of highest analytical quality and purity. Leaves of the *Grewia asiatica* (plant) were collected in May from agricultural department of Bahawalpur. The metal used in this experiment were chloroauric acid ($HAuCl_4{\cdot}3H_2O$) from Merck. *2,2-diphenyl-1-picrylhydrazyl* (DPPH), Sodium Borohydride ($NaBH_4$), Ciprofloxacin and Methylene blue (MB) was purchased from local vendors. De-ionized water used for synthesis or solution preparation.

2.2 Preparation of plant extract

GALE was rinsed firstly with simple water and then de-ionized water to remove impurities. The extract of the leaves was prepared by taking 10g of leaf in 100ml of de-ionized water. To get the leaf extract the solution was heated at 90°C in the water bath for 20 minutes with stirring frequently and then allowed to cool at ambient temperature. The extract was taken and filtered. For future usage, the filtered solution was kept in a clean, dry beaker.

2.3 Synthesis of Optimized Gold nanoparticles

The 5ml solution of chloroauric acid (1mM) was boiled and 5ml of plant extract was added into it. The mixture was stirred for 2 minutes. The color of the solution appeared as ruby pink. In next 15 minutes, the color changed to violet. The change in color was the indication of preparation of gold nanoparticles. The resulting colloidal solution of AuNPs was then stored for further application and characterization. The steps for synthesizing gold NPs are depicted in Fig. 1.

2.4 Characterization of synthesized AuNPs

The primary methods used for characterization of synthesized nanoparticles include UV-vis spectroscopy, FTIR spectroscopy, SEM, EDX, and X-ray diffraction (XRD). A UV-Vis spectrophotometric investigation was carried out to evaluate the absorbance value of the nanoparticles in the region of 300–600 nm. By measuring SPR, this approach can validate the production of nanomaterials (Mahendran et al. 2022).

2.5 Degradation of Organic Dye

Plant synthesized GAAuNPs were used as a catalysts at room temperature for catalytic reduction of methylene blue (MB). To begin the catalytic degradation experiment, 2.5ml of MB dye solution (0.04mM) was added to a quartz cuvette with a one-centimeter path length. The dye solution was mixed with 0.5 mL of the newly made $NaBH_4$ (0.06 M) solution before adding 0.5 mL of colloidal AuNPs. The solution was then gently stirred, and UV absorption was taken at regular intervals. Another sample was created using the same process but without nanoparticles. This was used as a comparison to prove that nanoparticles' reduction was more significant. The following equation was used to measure variations in MB absorption after different intervals to track the dye's reduction reaction:

Degradation (%) = $100\,(C_0 - C)/C_0$

While C = the concentration of dye upon irradiation, C_0 = initial concentration of dye.

2.6 Antibacterial activity

We employed Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria from the University of Agriculture's Institute of Microbiology in Faisalabad, Pakistan, to assess each sample's bactericidal efficacy. Bacteria were cultivated overnight in nutrient agar (Oxoid, UK) at 37°C. Disc diffusion method measured antibacterial activity. Briefly, 100 µL of the investigated microorganism suspension with 10^7 CFU/mL of bacterium cells on a nutrient agar medium was used. Agar plates already inoculated with the tested microorganisms were placed on top of the filter discs (6 mm in diameter), each of which had been individually impregnated with a compound solution. Ciprofloxacin (30 µg/dish) was utilized as a positive reference for bacteria to examine the sensitivity of strains/isolates in the analyzed microbial species. Negative controls were sample-free discs. The plates were placed for 2 hours at 4°C and then incubated at 37°C for 18 hours to accelerate the bacterial growth. Finally, the antibacterial proficiency was assessed by comparing the size of the growth inhibition zones (using a zone reader) (Shahid et al. 2021; Shakil et al. 2022).

2.7 Biofilm inhibition

The biofilm inhibition efficiency of plant extracts and prepared NPs was also assessed and detailed as reported by Perveen et al (Perveen et al. 2021). A sterilized 96-well plain plastic tissue cultivation plate was filled with 100 µL of dietary broth, 20 µL of injectable bacterial solution (Oxoid, UK), and 100 µL of the test sample to create the biofilm. Nutrient broth effectively became the sole item in the control wells. After the plates were introduced, they underwent an aerobic incubation at 37^0C for 24 hours. Each well contents had been thoroughly rinsed three times. The plates were rapidly agitated to remove any germs that were not adhering. The final adhering microbe was consistently detected using 220 microliters of 99% methanol, and the plates were flattened and left to dry for 15 minutes. After 5 minutes, the plates were dyed by pouring 220 mL of 50% crystal violet into each well. To remove the further discoloration, the plates were cleaned. The plates were air-dried; 220 µL of 33% (v/v) glacial acetic acid were added to each well to once again dissolve the dye that had been attached to the adherent cells. At 630 nm, the optical concentration of each well is carefully measured with a micro plate reader (Qasim et al. 2020). The findings of the three examinations of each bacterial strain were averaged. The following expression is used to determine the % of inhibition of growth of bacteria.

$INH\ \% = 100 - (OD_{630\ sample} * 100) / OD_{630\ control}$

2.8 Antioxidant activity by DPPH assay

As described by Siddiqui et al., the radical scavenging capacity of plant extracts and prepared NPs was evaluated using DPPH. (Siddiqui et al. 2020). For the antioxidant experiment, 3ml of plant extract was mixed with 1ml of freshly prepared 0.004% DPPH in methanol. The mixture was subsequently placed in a dark area. Then, the absorbance change of the solution was observed at 517 nm. Strong radical scavenging interest exists for reaction aggregates with low absorbance. Additional research was done on the antioxidant activities of butylated hydroxytoluene (BHT) and ascorbic acid. An alternative solution without plant extract was obtained as a control. There have been duplicate runs of every experiment. The IC_{50} value was calculated from the graph of inhibition versus sample concentration.

3. Results and Discussion

3.1 UV-visible spectroscopy study of the synthesis of gold nanoparticles

The gold nanoparticles were synthesized using the biogenic method in the present study. The formation of synthesized gold nanoparticles was verified by scanning the colloidal solution at various time intervals using a UV-VIS spectrophotometer at wavelengths ranging from 350 to 700 nm. The reduction of metal ions after an interaction with *G. asiatica* leaf extract (GALE) and the emergence of a ruby red color visibly demonstrate the creation of gold nanoparticles (GAAuNPs). GAAuNPs exhibited two peaks with λ_{max} at 400 nm and 550 nm, as shown in Fig. 2a. Furthermore, the scanning of the colloidal solution was repeated four times at regular intervals, but no change in absorption spectra appeared, confirming the successful formation of GAAuNPs. Next, the optimized conditions for the synthesis of the GAAuNPs were analyzed. Gold nanoparticles were synthesized using metal salt and extract in 1:1, 1:2, 1:3, and 1:4 ratios. The absorption showed that the optimum concentration for synthesizing GAAuNPs was 1:1 (Fig. 2b). Similarly, AuNPs were synthesized using different concentrations of precursor metal salt ranging from 1–4 mM. At 1mM concentration of precursor metal salt, the synthesized nanoparticles yielded an intense SPR band, indicating 1mM as the optimum concentration for synthesizing GAAuNPs (Fig. 2c). Bernawi et al. reported this type of UV-visible absorption spectra for the biosynthesized gold nanoparticle (Barnawi et al. 2022).

3.3 FTIR Spectroscopy

To analyze the FT-IR spectra of biosynthesized AuNPs, the KBr technique was used. The process involved mixing the AuNPs with KBr under high pressure to create a slice for scanning between 400 and 4000 cm^{-1}. The FTIR spectra of leaf extract and produced gold nanoparticles exhibited a broad band at 3374cm^{-1}, corresponding to OH group(Sher et al. 2021b). The weak peaks at 2363cm^{-1}, 2068cm^{-1} and the strong band at 1650,1380 and 1112 cm^{-1} in AuNPs indicate the characteristics of alkynes (C) and carbonyl groups like C = O, COOH groups, respectively (Muniyappan and Nagarajan 2014; Thangamani and Bhuvaneshwari 2019). These peaks result from the extract's soluble organic components, which may be involved in producing gold nanoparticles (Fig. 3).

3.4 X-ray Diffraction Analysis

A technique for analyzing the size, dimensions, and composition of nanoparticles is called X-ray diffraction spectroscopy. By using XRD analysis, Au NPs' purity and crystallinity were validated. Figure 4 displays the XRD pattern of GAAuNPs. XRD of GAAuNPs yielded four diffraction peaks at angles of 38.29°, 44.63°, 64.95°, and 77.91° corresponding to diffraction planes of (111), (200), (220), and (311) (Devi et al. 2015; Han et al. 2017). XRD on Au NPs reveals that the particles have a face-centered cubic structure, which matches the JCPDS card number 04-0784, and the pattern is consistent with previous publications(Kulkarni et al. 2019; Ramesh et al. 2019). The presence of four peaks specific to Au NPs', as shown in Fig. 5, indicates the purity of the particles. The Debye-Scherrer equation was used to calculate crystallinity, representing $D = k\lambda/\beta \cos\theta$ where D is the calculated average crystalline size. The average crystallinity of biosynthesized particles was found to be8 nm.

3.5 SEM-EDX analysis

The shape and content of GAAuNPs were investigated using scanning electron microscopy combined with energy-dispersive X-rays (SEM-EDX). The GAAuNPs were aggregated and represented in SEM (Fig. 5a). An EDX analysis was conducted to ascertain the GAAuNPs' elemental makeup. The metallic gold was observed at 2.2 keV, the highest percentage in EDX. In comparison, carbon and oxygen were situated at 0.25 and 0.45 Kev, confirming the organic moiety bound to the gold metal nanoparticle (Fig. 5b). In EDX spectra, strong Au and O peaks show that gold nanoparticles have formed and are very pure (Fig. 5b).

3.6 Degradation of Methylene Blue

MB, one of the most widely used organic dyes, is harmful in wastewater. in the presence of NaBH4. In the current work, the catalytic performance of biosynthesized gold nanoparticles (GAAuNPs) was checked against MB in the presence of $NaBH_4$ (reducing agent). A control sample containing $NaBH_4$ only (without GAAuNPs) was also run to check the catalytic effect of green synthesized AuNPs. A UV-visible spectrophotometer was used to measure changes in MB absorption to track the dye degradation process by gold nanoparticles. Figure 6b show that $NaBH_4$ and green-fabricated AuNPs effectively degraded MB by reducing its absorption peak at 664 nm. The presence of Au nanoparticles triggered the breakdown of methylene blue, resulting in complete degradation within 8 minutes. This indicates that the AuNPs were effective in catalyzing the reduction of MB. The control sample ($NaBH_4$ solution without AuNPs) experienced a slow MB degradation of 15 minutes. Thus, AuNPs acted as excellent catalysts and improved MB degradation (Fig. 6a).

3.7 Analysis of Biofilm inhibition

The GALE and GAAuNPs were subjected to biofilm inhibition assays against *S. aureus* and *E. coli.* The GALE showed 42.76% inhibition against *E. coli* and 7.22% inhibition against *S. aureus*. In contrast to GALE, the GAAuNPs were more vigorous against both strains and inhibited 55.04% *E. coli* and 46.12% *S. aureus.* The biofilm inhibition of GAAuNPs was better than plant extract for both strains as shown in Fig. 7a. The biosynthesized nanoparticles had an IC_{50} of 0.090 against *E. coli* and 0.10 against *S. aureus*. These values were similar to the standard (ciprofloxacin) IC_{50} values of 0.068 against *E. coli* and 0.078 against *S. aureus* (Fig. 7b). Consequently, it is anticipated that the GAAuNPs that were synthesized will be able to inhibit biofilm formation in both *E. coli* and *S. aureus*. Similar results have been reported by previous researchers (Manandhar et al. 2019; Hasan et al. 2020).

3.8 Antioxidant activity by DPPH assay

The antioxidant activities of GALE and synthesized GAAuNPs were tested using the DPPH assay. The results are expressed in IC_{50} g/mL, with ascorbic acid serving as the standard. The synthesized GAAuNPs showed more significant antioxidant activity than the standard control (ascorbic acid) (Fig. 8a). The maximum activity of GAAuNPs may be attributed to the presence of diverse functional groups, as demonstrated in FTIR (Fig. 3) (El-Sheekh and El-Kassas 2016). The findings suggest that GAAuNPs may be used in place of antioxidants to treat diseases brought on by free radicals. In order to engage with free radicals and convert them into more stable molecules that can halt a chain reaction, GAAuNPs operate as electron donors (Siddiqi et al. 2018). AuNPs made from plant extracts exhibit powerful antioxidant capabilities, according to a number of prior investigations (Dorosti and Jamshidi 2016; Vimalraj et al. 2018). The IC_{50} values of leaf extract and AuNPs were 1.24mg/ml and 0.6mg/ml, respectively. Therefore, it is concluded that the biosynthesized GAAuNPs free radical scavenging activity was enhanced compared to the leaf extract.

3.9 Antibacterial Activities

Several studies have discovered that AuNPs are more valuable and effective in biological applications than conventional metal NPs (Akintelu et al. 2020; Hammami and Alabdallah 2021). Because they have a large surface area to interact with the bacterial cell wall, three-dimensionally structured nanoparticles can quickly lead to structural cell deformation (Du et al. 2014). The GAAuNPs were subjected to antibacterial activity utilizing bacterial strains; *S. aureus* and *E. coli* and ciprofloxacin as a reference standard. The synthesized nanoparticles and leaf extract inhibited two bacterial strains, *S. Aureus* and *E. coli* (Fig. 9b).

The GALE showed a 12mm and 9mm inhibition zone against *E. coli* and *Staphylococcus*, while the GAAuNPs showed a 12mm and 13mm zone of inhibition against both *E. coli* and *Staphylococcus*. In this experiment, the GALE had enhanced activity compared to derived nanoparticles. The zone of inhibition for both bacteria was represented graphically in Fig. 9b. The current research has demonstrated that these nanoparticles can combat human diseases because they are effectively absorbed by the peptidoglycan-based cell walls of bacteria. Their small size promotes optimum absorption, ultimately killing the germs (Qamar et al. 2017; Khalil et al. 2020). According to research, large amounts of hydrogen peroxide and other reactive oxygen species can disrupt protein function by damaging bacterial DNA and cell membranes (Qamar et al. 2022a; Javed et al. 2023). According to the results, nanoparticles are a helpful resource for combating human infections.

Conclusions

The current work uses GALE to make GAAuNPs straightforwardly and safely. The characterization investigations disclosed the structural aspects and the function of the stabilizing agents during the synthesis of AuNPs. The successful synthesis of AuNPs was verified by a colour shift from light yellowish to ruby red and by scanning via UV-VIS spectroscopy during production. FT-IR investigations have shown the characteristics of alkynes (C), carbonyl groups, and other chemicals in the *G. asiatica* leaf extract that are responsible for converting Au^{+3} ions into GAAuNPs. The SEM-EDX analysis showed nanoparticles that were pure and formed in clusters. The synthetic GAAuNPs used as catalysts showed outstanding methylene blue degradation efficiency. The dye was destroyed in just six minutes. The efficiency of GAAuNPs in degrading the dye was even higher than in previously published studies. The GAAuNPs produced displayed significant biofilm inhibition and antioxidant and antibacterial activity. According to the findings of the study, GAAuNPs can be employed as catalysts and biologically active substances for environmental remediation applications.

Declarations

Ethics. No ethics approval is necessary since neither humans nor animals were used in the study.

Author contributions. S.M.: writing—original draft, formal analysis, methodology; N.A.: investigation, project administration, supervision and validation; M.A.Q: writing—review and editing; A.F, S. N. P: conceptualization, formal analysis, and methodology; A. Y: validation and writing—review and editing; A. N. M. A. A..

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Figures

Figure 1

Synthesis steps of Au NPs.

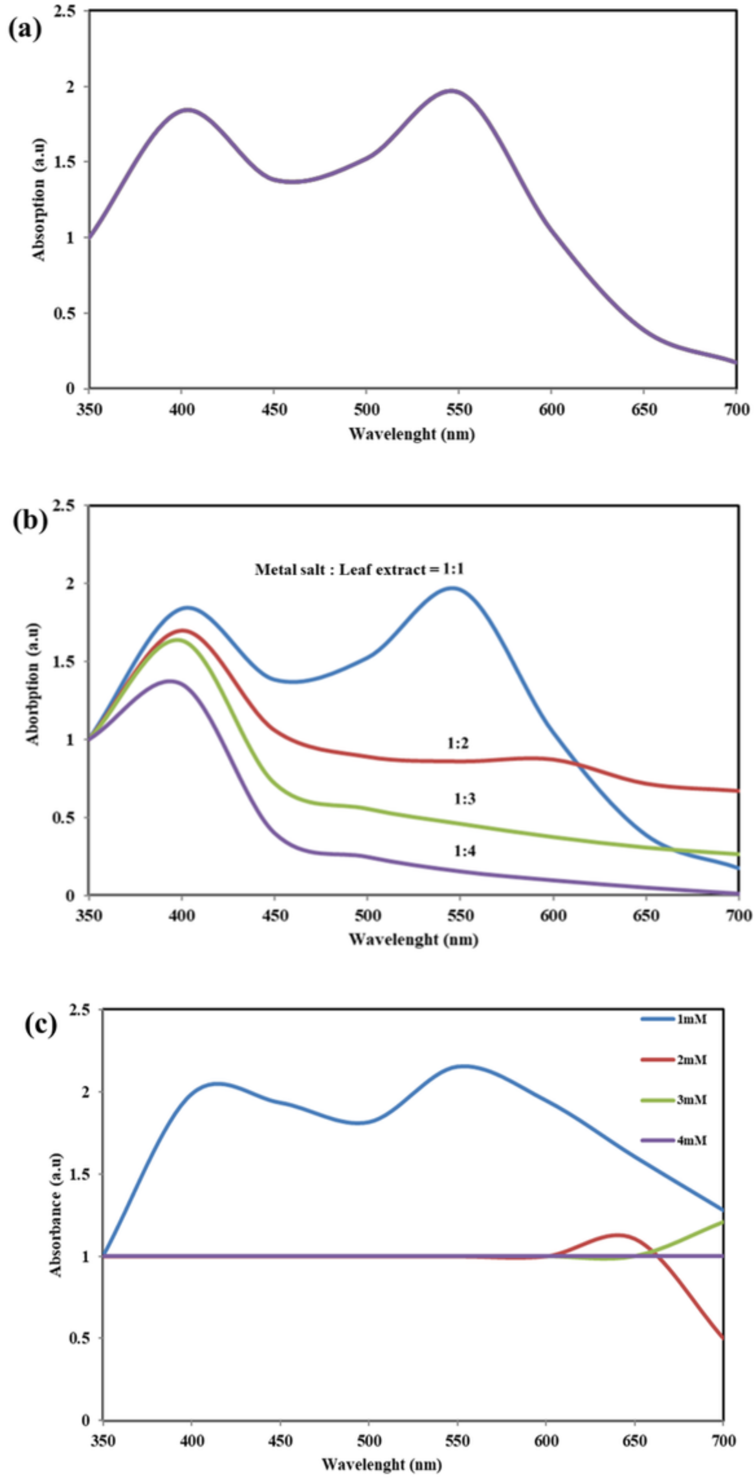

Figure 2

UV-VIS absorption spectra (a) effect of change of metal salt and plant extract ratio (c) effect of change in metal salt concentration on the synthesis of gold nanoparticles using *Grewia asiatica*leaf extract.

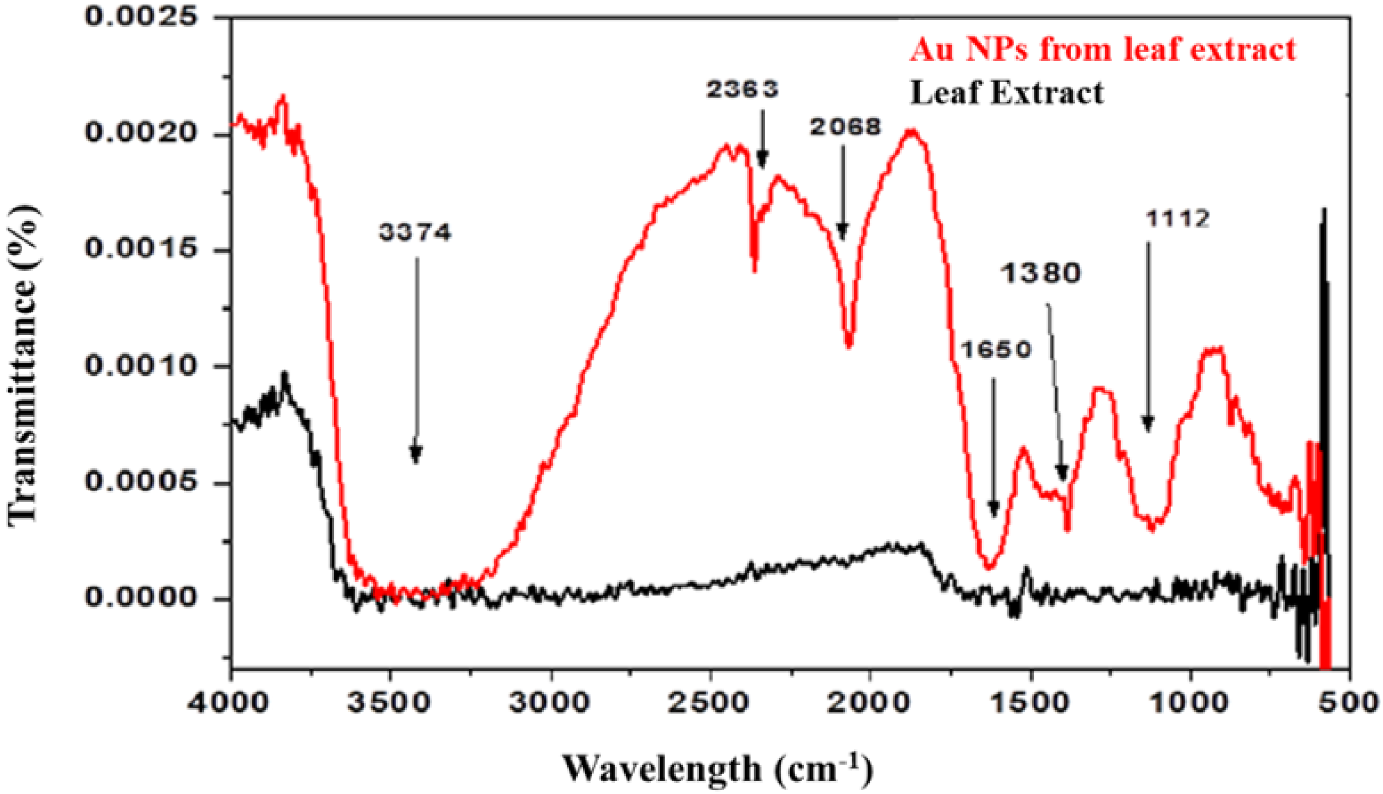

Figure 3

FTIR analysis of gold nanoparticles synthesized from *G.asiatica* extract

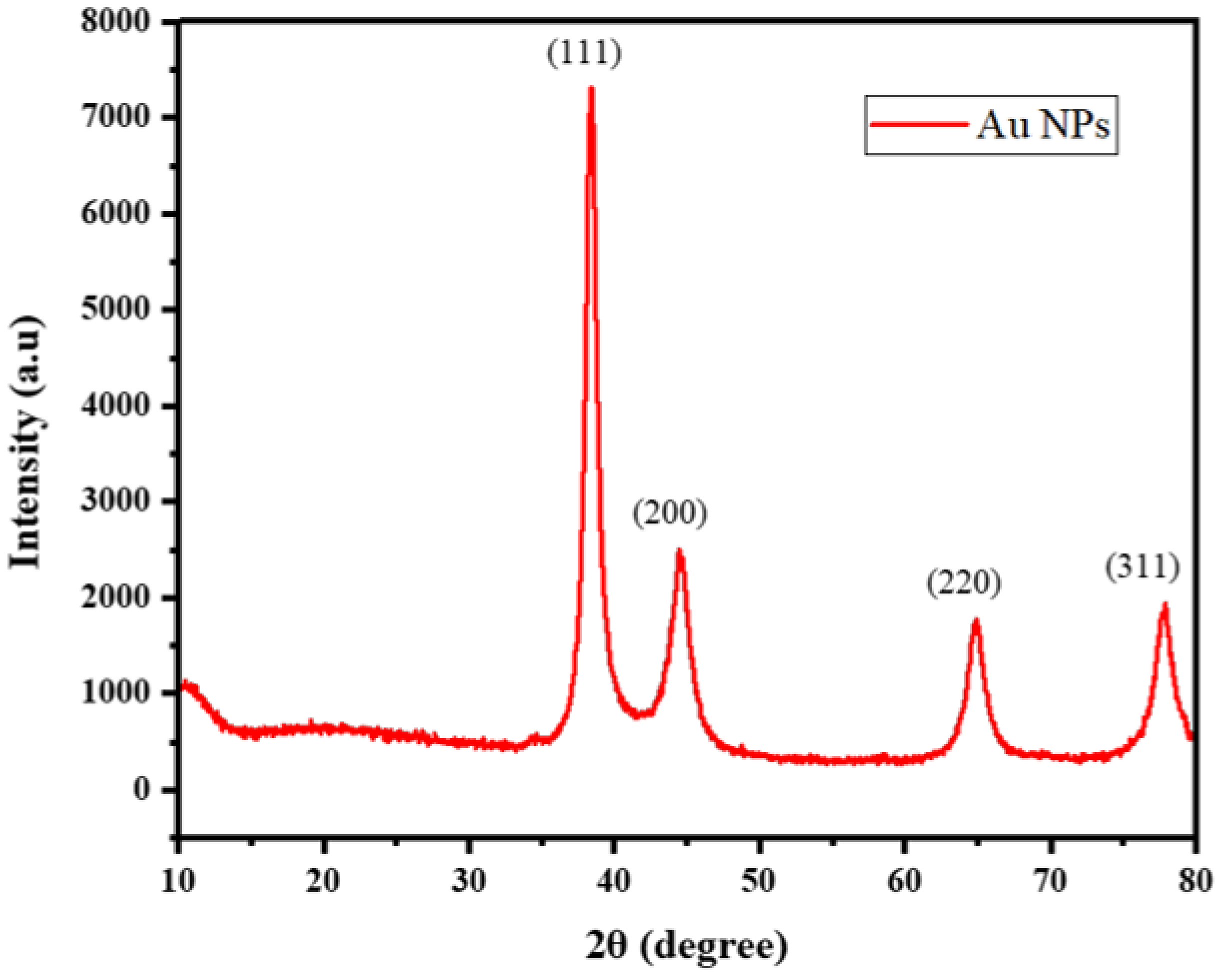

Figure 4

Synthesized gold nanoparticles' X-ray diffraction pattern

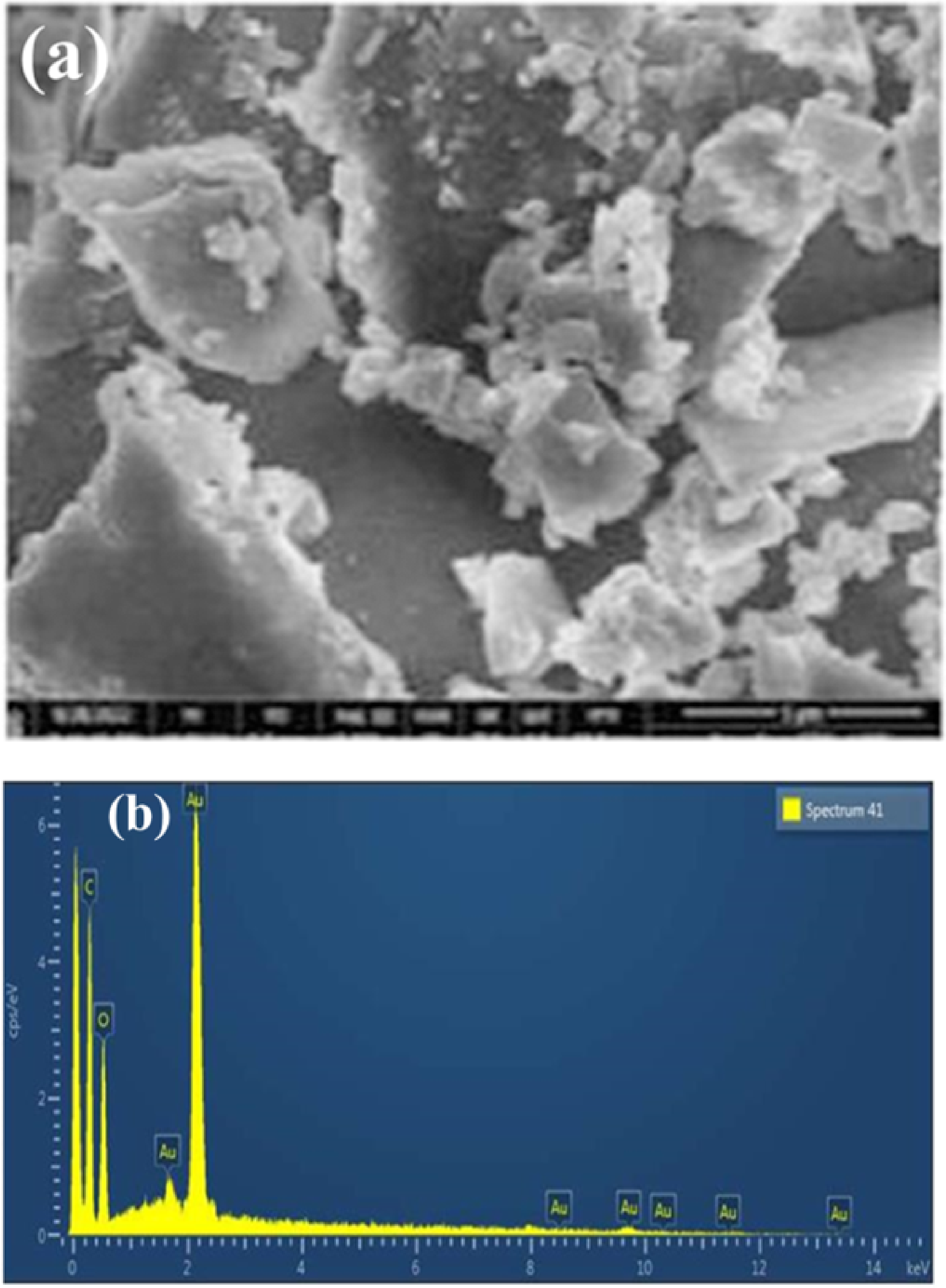

Figure 5

SEM (a) EDX analysis of Au nanoparticles (b).

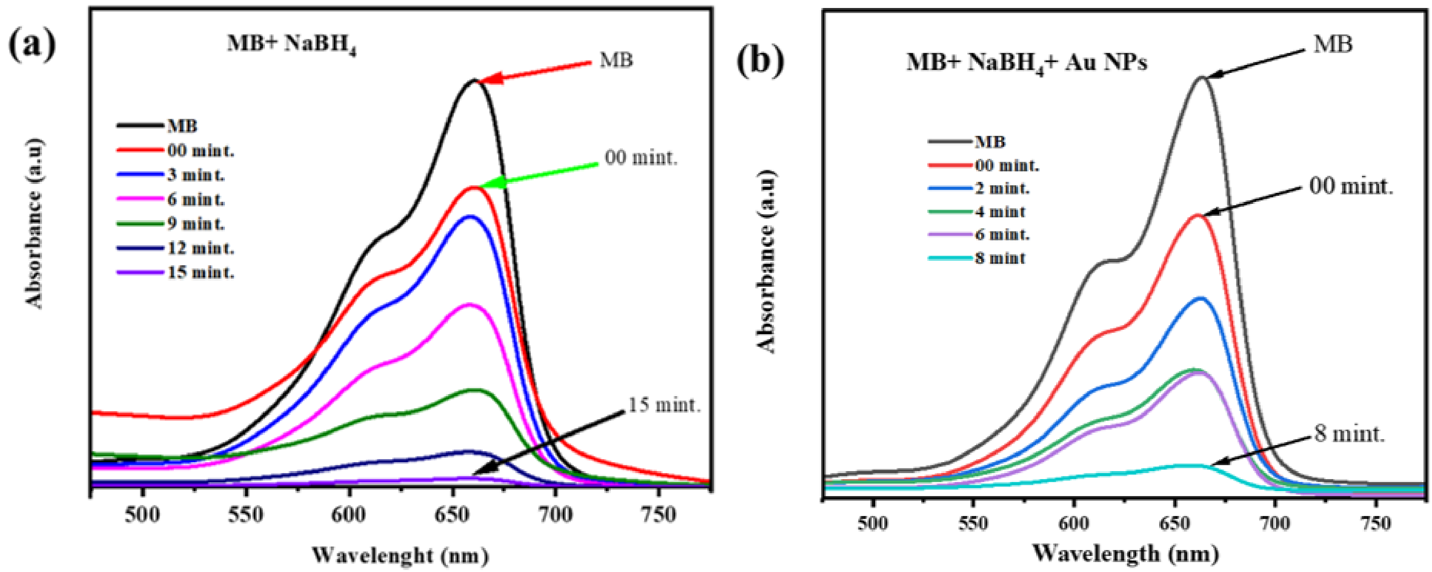

Figure 6

Degradation of MB in the presence of $NaBH_4$ (a) and in the presence of $NaBH_4$+AuNPs (b).

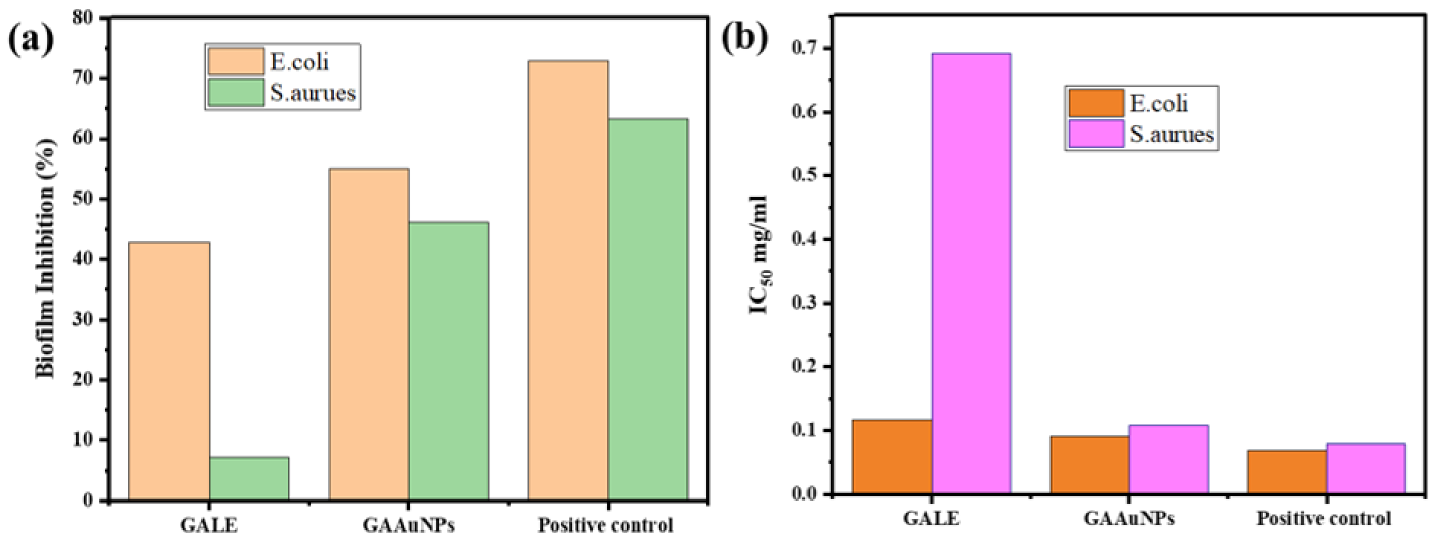

Figure 7

Biofilm inhibition (%) (a) and IC_{50} values for Biofilm inhibition by gold nanoparticles (b).

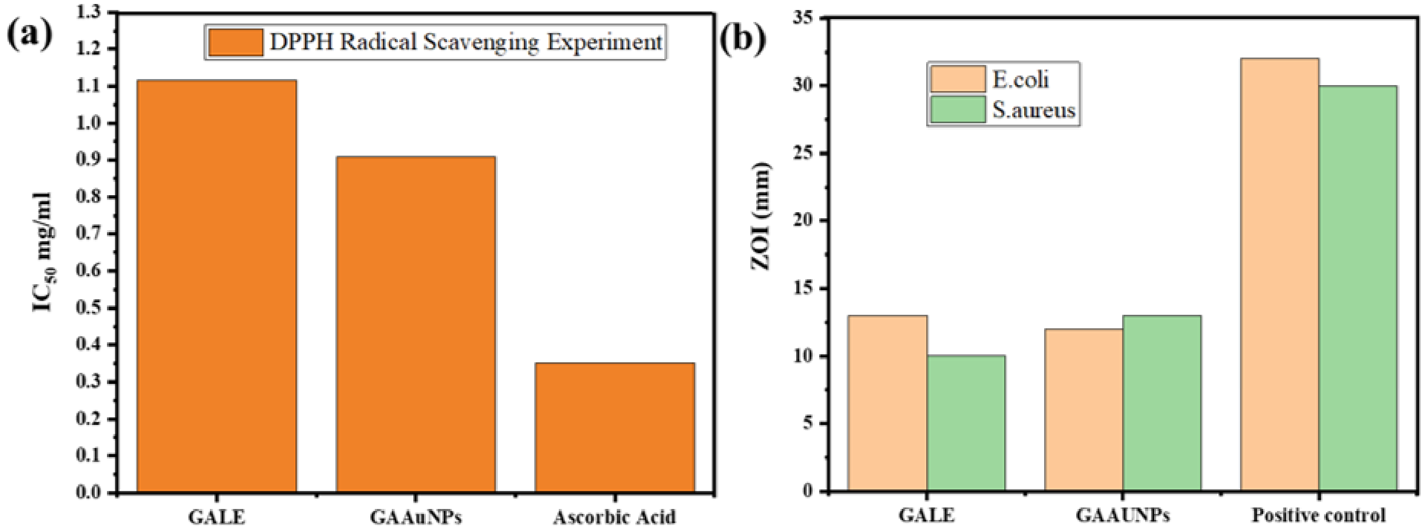

Figure 8

DPPH scavenging activity (a) and Antibacterial activity of Au nanoparticles (b)