Facile green Synthesis and Characterization of Gold Nanoparticles with Gum Ghatti (Anogeissus latifolia); Catalytic Reduction of Rhodamine 6G, Scavenging and Anti-Microbial Activity

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Abstract- This paper reported the outcomes of a facile and active Gold nanoparticle synthesis approach based on aqueous solution of gum ghatti and $HAuCl_4$, without addition of any hazardous chemical for the reduction and capping agent. However, formed effective stabilization of Gold nanoparticles in gum ghatti (AuNPGT) with a fine size and average diameter was 5±2 nm. The successful synthesis of AuNPGT was confirmed by UV–visible spectroscopy, Fourier transform infrared spectroscopy, X-ray powder diffraction, Transmission electron microscopy, EDS and DLS. The conformed AuNPGT were purple in color and characterized by a peak at 525–530 nm in the UV–Vis spectrum. The catalytic activity of microwave assisted AuNPGT was examined with Rhodamine 6G. The AuNPGT exhibited excellent catalytic activity and the reaction showed pseudo-first order with rate constant was 0.103287 min^{-1}. AuNPGT was showed highly radical scavenging activity against to DPPH and ABTS, respective IC_{50} values were 31.56 µg/mL and 40.2 µg/mL. AuNPGT exhibited significant anti-microbial activity towards the Gram positive and Gram negative bacteria such as Streptococcus aureus and Escherichia coli.

Index Terms- Green synthesis, Gold Nanoparticles, Microwave assisted, Catalysis, Scavenging activity, Anti-microbial.

I. INTRODUCTION

Noble metal nanoparticles have an extremely high surface-to-volume ratio and high stability of atoms at their surfaces and are known to exhibit high catalytic performance in a variety of preparative chemical transformations (Rodrigues et al 2019). These emergent properties have the potential for great impacts in optical, electronics, mechanical, biomedical, catalysis and other fields (Zhu et al. 2016; Iravani et al. 2011; Costa et al. 2014). Some examples are the reactions like hydroformylation, Fischer-Tropsch, and isomerization etc., which also use nanoparticles like Ir, Au, Rh, Pt, and Pd as catalysts (Liu et al. 2018). These dimension-dependent properties are widely believed to be a result of the high ratio of surface to small atoms as well as the bridging state between atomic and bulk materials (Costa et al. 2014). However, as nanoparticles are intrinsically metastable and tend to either irreversibly aggregate or leach atoms of metal under the conditions of standard chemical processes (Jeevanandam et al 2018).

In the recent decade, Gold nanoparticles (AuNPs) are important because of shape, size, crystal structure arrangement, most stable mono-metallic nanoparticles and excellent catalyst in redox reactions and synthesis of fine chemicals especially in selective oxidation owing to their large surface area (Serena et al 2001). Past studies have shown that from gold salts by different stabilizing and reducing agents such as PVA (Khanna et al. 2005) PVP (Muntuwenkosi et al. 2011), Thiol (Muriel et al. 2011) and N,N-dimethylformamide (Esumi et al. 2000). However, these chemicals are very reactive and pose a potential ecological hazard and biological risks after treating. Novel fundamental, applied boundary in material science, and such as Nanobiotechnology (Fakruddin et al. 2012), Bionanotechnology (Ziad et al. 2012), these endeavors goal at the total elimination or at least the reducing the generated waste and the execution of ecological processes.

Recently, the green chemistry which targets to reduce or remove substances hazardous to human health, here design, development, implementation of chemical processes and products are becoming more important to the environment (Kralisch et al. 2015; Raveendran et al. 2003). The green synthesis of metal nanoparticles (NPs) involve made a broad spectral progress in recent times due to their unique chemical, optical, magnetic and electrical properties (Kalpana et al. 2018).

In the present study Gum ghatti (GT) used as reducing and stabilizing agent to AuNPs, GT also termed as "Indian gum", is a tree exudate attained from Anogeissus latifolia wall as a plant maker of polymer, flexible for biodegradation (Takao et al.). GT composed of sugars as D-mannose, D-xylose, D-glucuronic acid, D-rhamnose, D-glucuronic acid, D-glucose, L-arabinose, L-fructose, manose, xylose, and aspartic acid (Aruna et al, 2012). As a plant producer of polymer, flexible for biodegradation, this synthesis was carried out in an aqueous medium by microwave oven at 550W for 7 min, it is facile and green simplistic synthesis of AuNPGT. Additionally, the catalytic activity of microwave assisted AuNPGT towards to Rhodamine 6G has been examined by spectrophotometric method. Rhodamine 6G dye belongs to a branch of dyes termed as xanthene dyes, extremely high fluorescent dye in Rhodamine family is an organic pollutant and

cationic dye, highly hazardous to environment and their impact existing in industrial wastewater (Zhang et al. 2009). In this paper study has been made to revel the antioxidant activities of AuNPGT by using widely established free radical scavenging model system i.e., 2,2-diphenyl-1-picryhydrazyl (DPPH) and and 2,2'-azinobis-(3-ethylbenzothiazoline-6)-sulfonic acid (ABTS) have been studied by UV-Vis spectroscopy. Bacterial drug resistance worldwide has become a serious threat, minimizing the effective antibiotic options; therefore, innovative methodologies that improve antimicrobial action are urgently needed (Aslam et al. 2018). Agar disc diffusion technique studied against Streptococcus aureus (S. aureus) and Escherichia coli (E. coli).

Experimental

Materials

Auric Chloride Trihydrate $AuCl_3.3H_2O$, Rhodamine 6G, Sodiumborohydride ($NaBH_4$), DPPH, ABTS, Potassium persulphate and Ethanol purchased in Sigma-Aldrich. Gum ghatti, was purchased from Girijan Co-operative Corporation Ltd., Hyderabad, India and throughout the reaction used Milli.Q water.

Facile green Synthesis of AuNPGT

About 30 ml (0.5 to 0.1 mM) of aqueous solution $HAuCl4.3H_2O$ was taken in a 50 ml beaker and added 10 ml of (0.1 to 0.5%) aqueous solution of GT. The synthesis was proceeded in a microwave oven at a 550 W for 7 min the reaction was examined by UV–Vis spectrometry.

Procedure for reduction of Rhodamine 6G dye

AuNPGT was examined as a catalyst in the Rh6G dye reduction using AuNPGT. The reduction of Rh6G dye solution was passed out in a quartz cuvette and monitored using UV-Vis spectroscopy at room temperature and 0.5 ml of 1mM $NaBH_4$ was added to 2 ml of $1x10^{-5}$M Rh6G dye solution in a cuvette and the UV-Vis spectra was recorded at different time intervals (0 to 2 hours). Another set of the above solution 20 µL of 0.5 mM AuNPGT added and the progress of the reaction was subsequently followed at different time intervals (0 to 12 min) by UV–Visible spectrophotometer. Changes in the absorbance of the Rh6G solution was observed by examining the variations in the maximal absorption of the R6G solution at 527 nm.

Free radical scavenging activity of AuNPGT

Radical scavenging activity of microwave assisted green synthesized AuNPGT was assessed through monitoring the capacity of quenching the stable DPPH free radical form to non-free radical form. DPPH (2, 2-diphenyl-picylhyrazyl) ethanolic solution was appearance an intense purple color and strong absorption peak at 517 nm (Amrutham et al. 2020) and increasing of time by a gradual color change to the DPPH solution from purple to colorless. A solution of DPPH 1 mg in 100 ml ethanol, from the stock solution 3.0 ml was added with various concentrations (10, 20, 30, 40 & 50 µg/mL) of green synthesized AuNPGT and GT. The reaction is examined by a UV-Visible spectrometer, Ascorbic acid was as a positive control and assessment was tested in triplicate and averaged. Radical Scavenging (% RSA) was calculated using resulting equation.

$$\%RSA = (A_{DPPH} - A_S)/ (A_{DPPH}) \times 100$$

The quantity of initial DPPH decrease in the concentration by 50 % is defined as IC_{50} and values were calculated by the linear regression study of dose–response curve plotted between % RSA and concentration. Where the A_{DPPH} is ethanolic solution of DPPH and A_S is after incubation of different concentrations of AuNPGT of DPPH solution.

ABTS radical scavenging activity

The ABTS radical scavenging activity method was adopted from the (Rameshkumar et al, 2012) of the microwave assisted green synthesized AuNPGT. The ABTS solution was prepared by mixing two stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulphate solution in equivalent amount and allowed to react for 16 hours at room temperature in the dark, the radicals were stable in this form for more than 48 hours kept in the dark at room temperature. 2 ml of the resulting solutions was permitted to react with AuNPGT for different concentration ranging from 10 to 50 µg/mL and after 30 min of incubation time at room temperature, the absorbance was 0.709 ± 0.025 at 734 nm with ethanol and after 7 min interval. The same was done for the GT and Butylated hydroxyl toluene (BHT) standard of different concentrations. The percentage (%) ABTS radical scavenging activity of AuNPGT was measured and compared with GT and BHT. The amount of inhibition capacity of ABTS by the AuNPGT was estimated from the following equation;

$$ABTS\ radical\ scavenging\ activity\ (\%) = [(A_{ABTS}-A_S)] / (A_{ABTS})] \times 100$$

Where A_{ABTS} is the absorbance of blank ABTS; A_S is the absorbance of ABTS + AuNPGT, every sample was assayed with triplicate and the average IC_{50} value was calculated.

Anti-Microbial activity of AuNPGT

The antibacterial activity of AuNPGT was related to their zone of inhibition studied against gram positive S. aureus and gram negative bacteria E. coli. Disc-assay has found to be a simple, low-cost and reproducible practical technique (Katas et al 2019) agar medium was prepared by taking yeast extract (0.5 gr), tryptophan (1 gr), sodium chloride (1 gr) and bacterial grade agar (2.5 gr) in 100 ml of distilled water. Then the agar medium was sterilized by autoclaving at a pressure of 15 psi and 120^0C temperature for 30 min. This medium was transferred into sterilized petri dishes in a laminar air flow. After solidification of the media, overnight culture of S. aureus (100 µl) and E. coli (100 µl) was spread separately on the solid surface of the media. Sterile discs were kept on these inoculated plates with the help of sterile forceps. Sample (10 µl) solutions were placed on these discs and were incubated at 37^0C for 24 h in a bacterial incubator. The inhibition zone that appeared around the disc was measured and recorded as the antibacterial effect of AuNPGT and GT.

II. Results and Discussion

UV-visible Spectral analysis of AuNPGT

Addition of $HAuCl_4$ solution to GT solution results in exhibiting the yellow color and was kept in Microwave oven at 550W, which turned to intense red color within 12 min, confirming the synthesis of AuNPGT. The UV-Vis absorption

spectra of AuNPGT synthesis against different $HAuCl_4$ concentrations (0.1-0.5 mM) with 0.5% GT are displayed in Figure 1 (a). It is observed that, as the concentration of $HAuCl_4$ solution increases, there is a progressive enhancement in the intensity of the Surface plasma resonance band (SPR), which indicates, the increased formation of AuNPGT. The absorbance maximum was observed in the range of 525–530 nm, which is characteristic of gold SPR (Yulizar et al. 2017). Figure 1 (b) shows the UV–Vis spectra of the synthesized AuNPGT with different concentrations of GT (0.1–0.5 %) with 0.5 mM HAuCl4 after 7 min of microwave time turned to purple color. As the concentration of GT increases, there is a progressive enhancement in the intensity of the SPR band, which indicates the increased formation of AuNPGT.

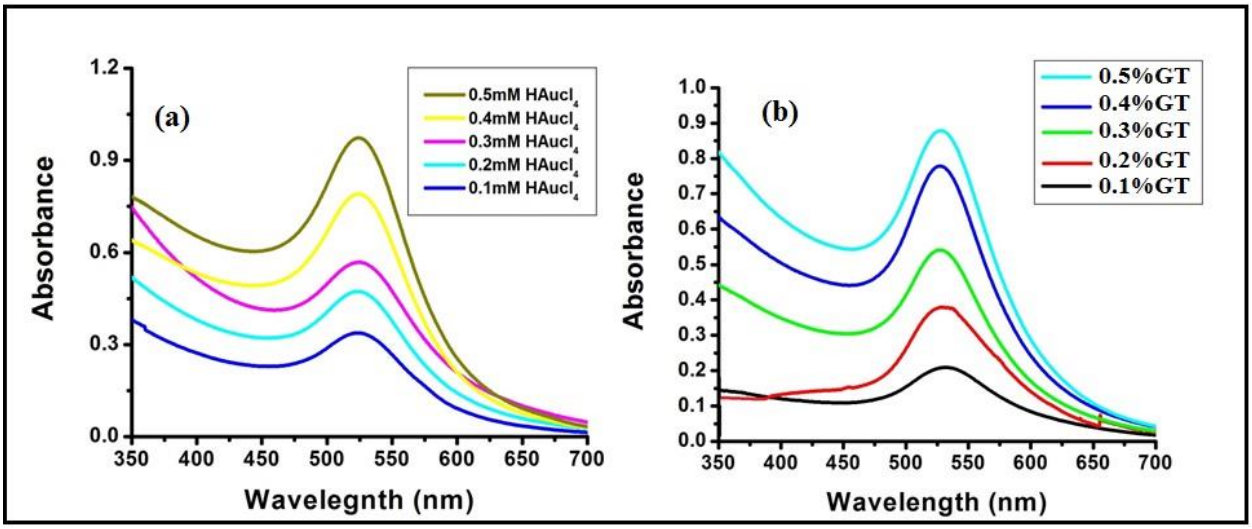


Figure 1. UV–Visible absorption spectra of AuNPGT **(a).** With various concentrations of $HAuCl_4$ (0.1 - 0.5mM) using (0.5%) gum ghatti **(b).** With various concentration of gum ghatti (0.1 - 0.5 %) using 0.5 mM $HAuCl_4$.

FTIR analysis of AuNPGT

FTIR spectrum shows possible biomolecules in GT responsible for the reduction and stabilization of AuNPGT. Figure 2 has shown bands at 3410, 2226, 1658, 1396, and 715 cm^{-1}. The broad band at 3410 cm^{-1} attributed to the –OH groups of the polysaccharides of the GT (Oueslati et al. 2020), a weak and broad peak at 2226 cm^{-1} and 1658 cm^{-1} peak represents C=O stretching and bend –NH of amide linkages. 1396 cm^{-1} corresponds to the C-O stretching vibrational frequency and sharp band at 715 cm^{-1} is responsible for bending vibration of $–CH_2$ group. AuNPGT showed a significant shift in the hydroxyl and carbonyl group bands. This is evident that the hydroxyl and carbonyl groups are responsible in the reduction and further stabilization process.

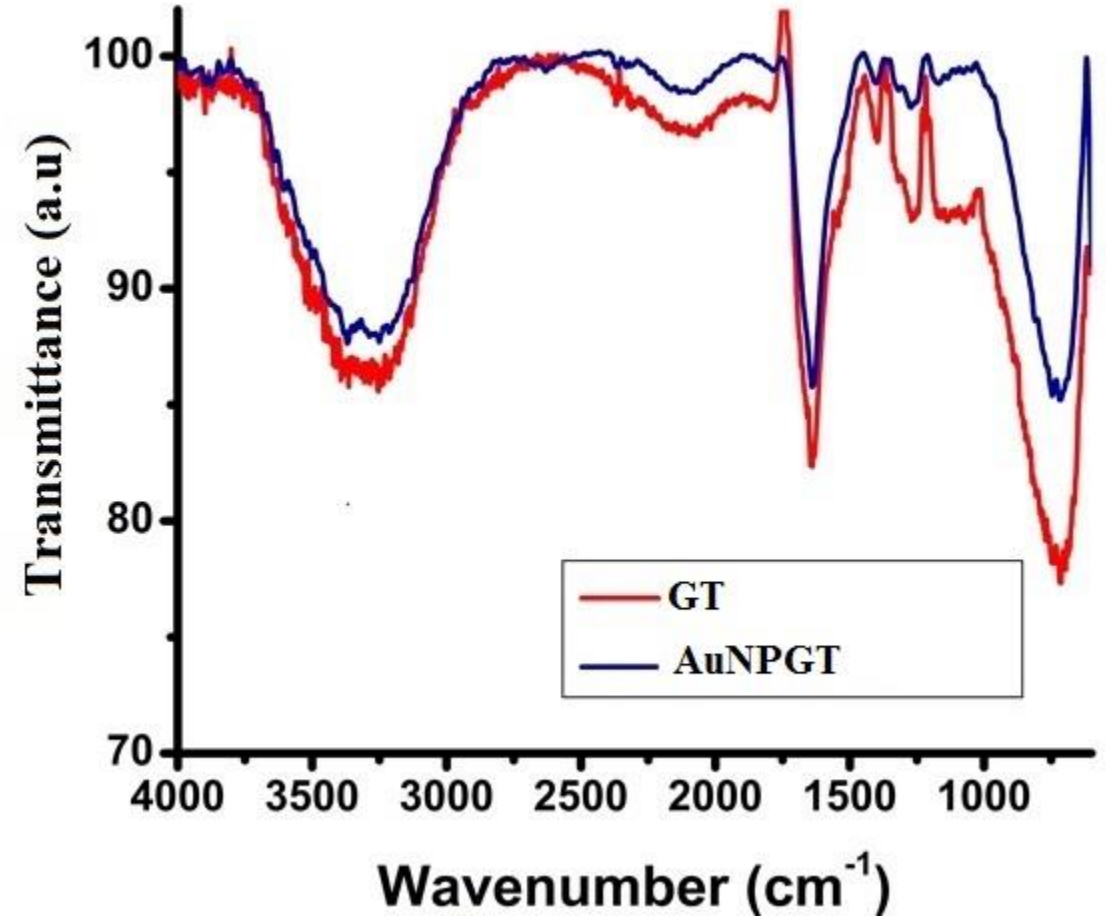


Figure 2. FT-IR Spectra of gum ghatti and AuNPGT.

XRD analysis of AuNPGT

Figure 3 shows the X-ray diffraction analysis obtained for AuNPGT synthesized using NG, the four different diffraction peaks at two theta values are 37.19, 44.40, 64.21 and 78.57◦, corresponding Bragg reflections are (111), (200), (220) and (311) (Nadagouda et al. 2014). Four sets of lattice planes are detected this may indexed of face-centered cubic (fcc) structure of AuNPGT. Crystalline in nature and spherical shape. The broadening of peaks indicating that AuNPGT were nanoscale in dimension.

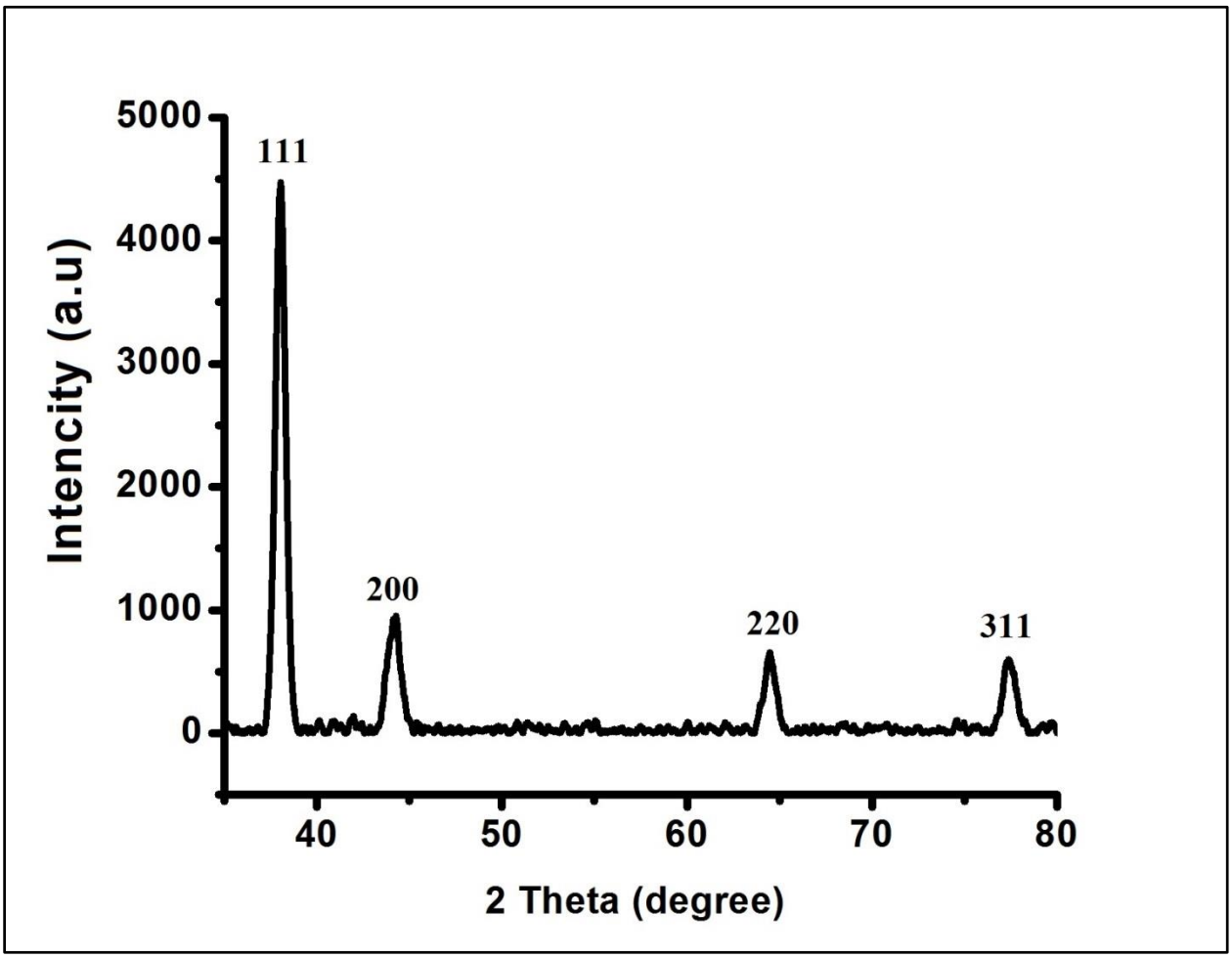


Figure 3. XRD pattern of the AuNPGT.

EDS

Figure 4 (a) EDS of AuNPGT has confirmed by the elements present in the gold nanoparticles, the optical absorption peak at 2.2 keV which was the pure Au of gold nanoparticles, occurrence of carbon and oxygen along with Au nanoparticles stabilized in biomolecule.

In the EDX spectra of AuNPGT, the presence of Cl^- signals indicated the presence of a minor amount of $AuCl4^-$ ions in the examined region.

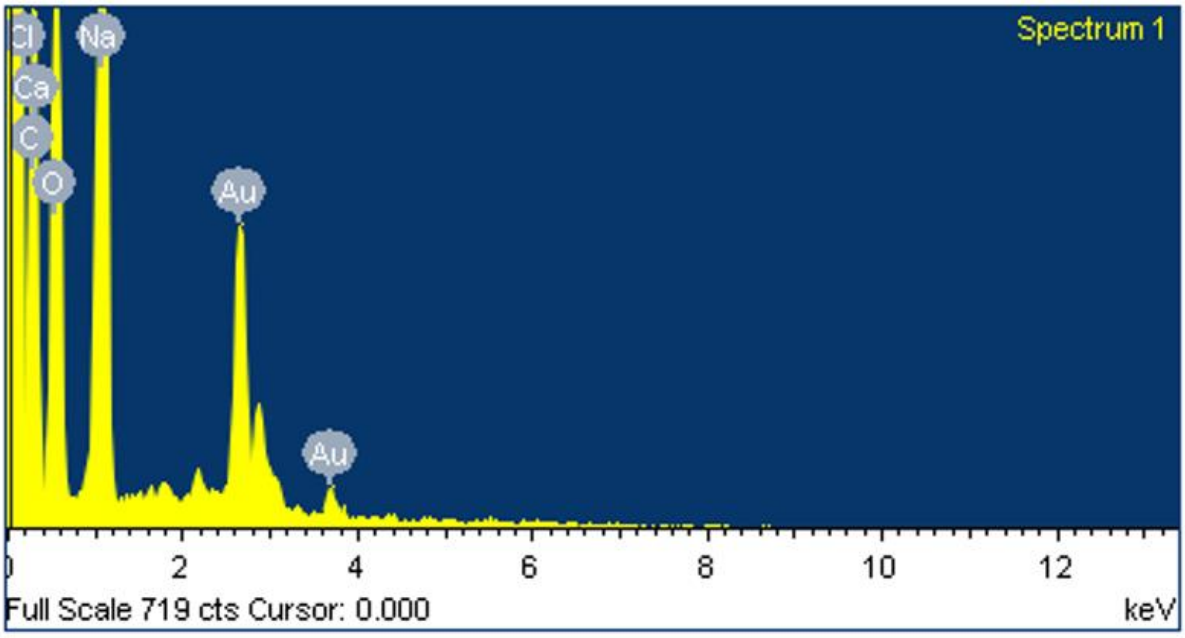


Figure 4. (a) EDS of AuNPGT.

TEM analysis of AuNPGT

Figure 5 (a) demonstrated the possibility of AuNPGT shape, size distribution, smooth surface morphology and a variable particle size. The spherical AuNPGT were formed with diameter ranging from 2 to 7 nm and are of highly mono-dispersed in nature. Histogram Figure 5 (b) was constructed by counting the size of AuNPGT, the average particle size distribution as 10 ± 2 nm.

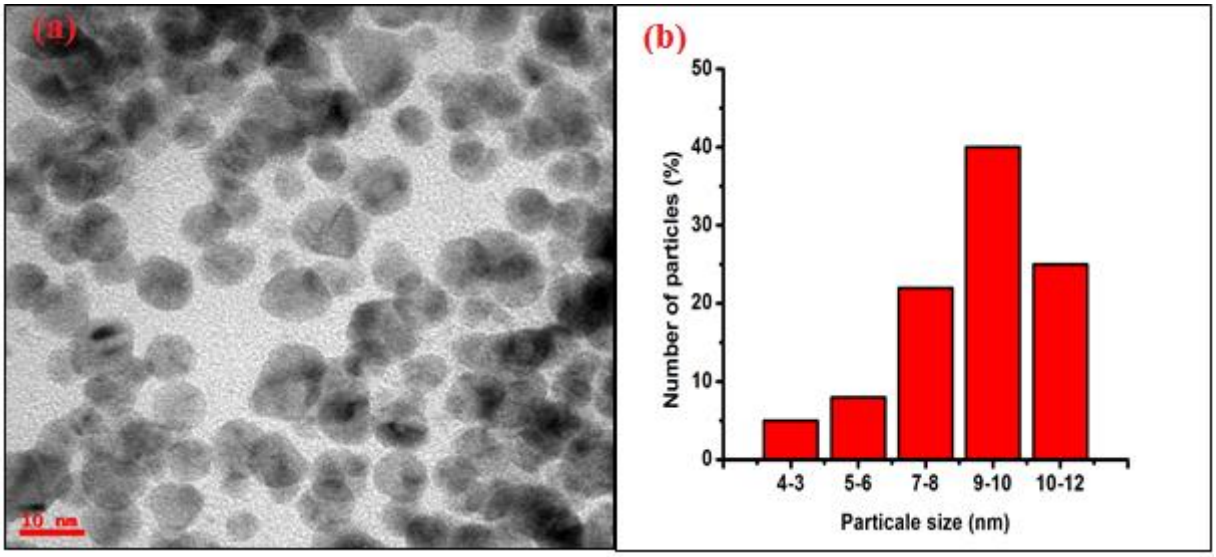


Figure 5. (a) TEM image of AuNPGT in aqueous system using gum ghatti as reducing and stabilizing agent. (b) Histogram showing the size distribution of AuNPGT.

Catalytic activity of Rhodamine 6G dye reduction

The catalytic activity of AuNPGT investigated by using the reduction of Rh6G dye, which was exhibited in the amount of $NaBH_4$ as an ideal system. 0.5 ml of 1mM $NaBH_4$ was added to 2 ml of $1x10^{-5}$M Rh6G dye in a cuvette and the UV-Vis spectra was recorded at different time intervals (0 to 2 hours). When a solution of $NaBH_4$ was mixed with a solution of Rh6G dye, minor color change was observed even after a prolong incubation of 2 hour as shown in Figure 6. To the another set of the above solution 20 µL of 0.5 mM AuNPGT added and the progress of the reaction was subsequently followed at 2 min time intervals by UV– Visible spectrophotometer.

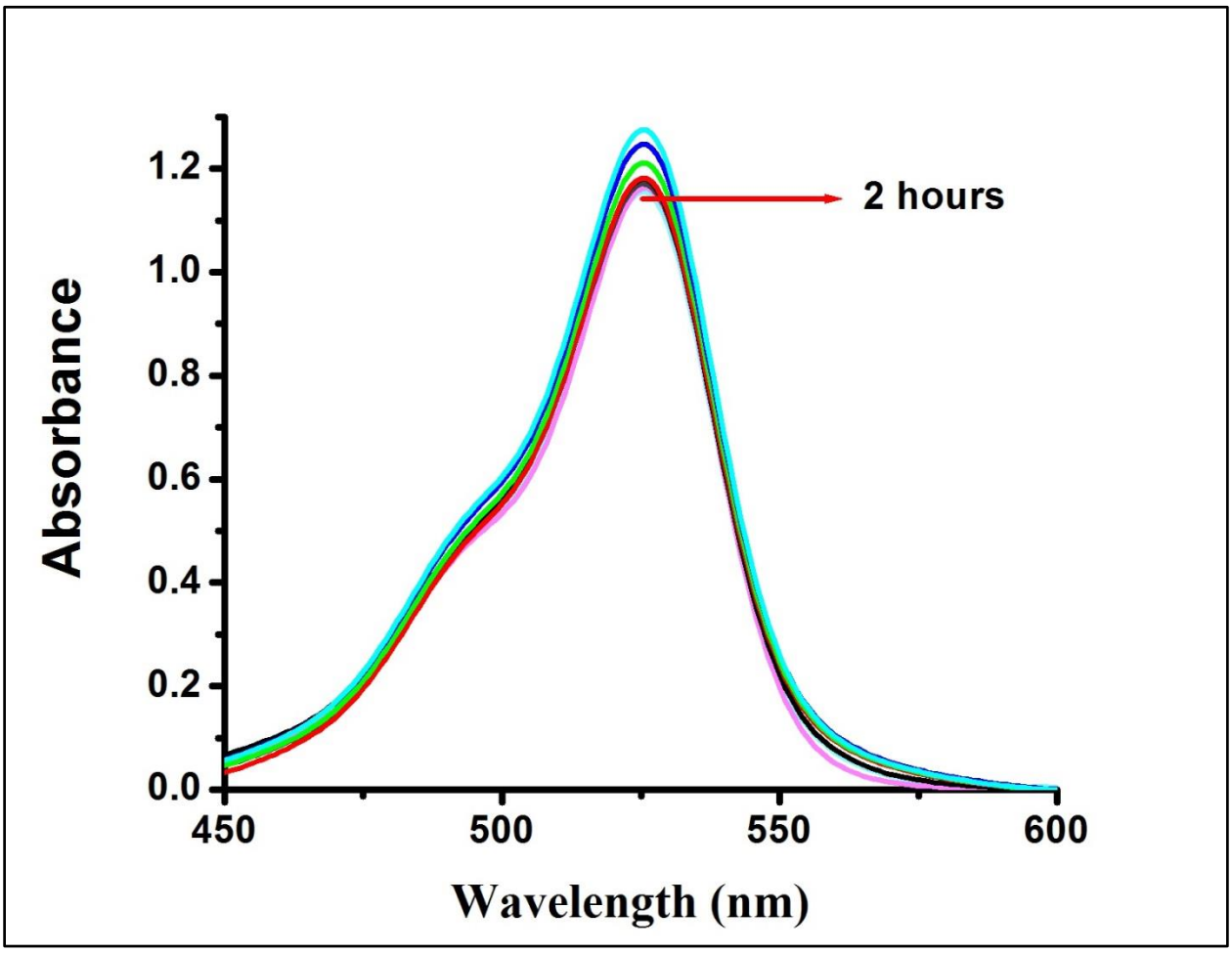


Figure 6. UV -Visible spectra of Rh6G in the presence of $NaBH_4$.

The complete reduction of Rh6G dye is accomplished in 12 min in the presence of AuNPGT shown in Figure 7 (a). The AuNPGT act as an electron initiative and electron change take place via AuNPGT from BH_4^- (donor) to Rh6G (acceptor) molecules. Hence, the AuNPGT prepared with GT proved to be outstanding catalyst for the reduction of Rh6G dye. Scheme 1 showed the mechanism of catalytic reduction of Rh6G. The linear relationship between ln (A_t/A_0) versus reduction time in minutes Figure 7 (b) this follows pseudo first order reaction kinetics with respect to Rh6G, reaction rate constant was found to be 0.103287 min^{-1}.

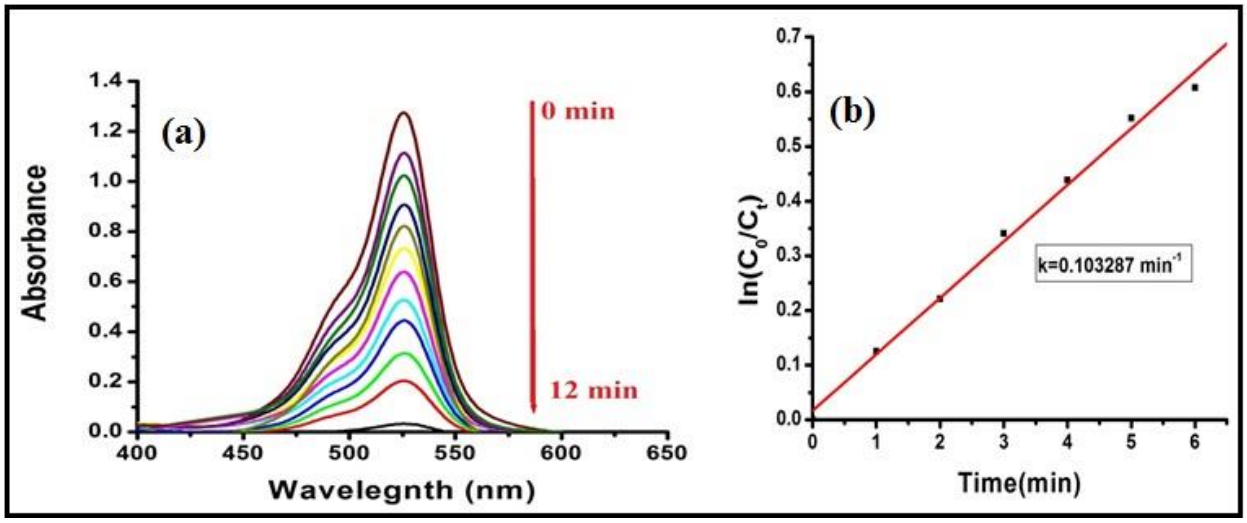
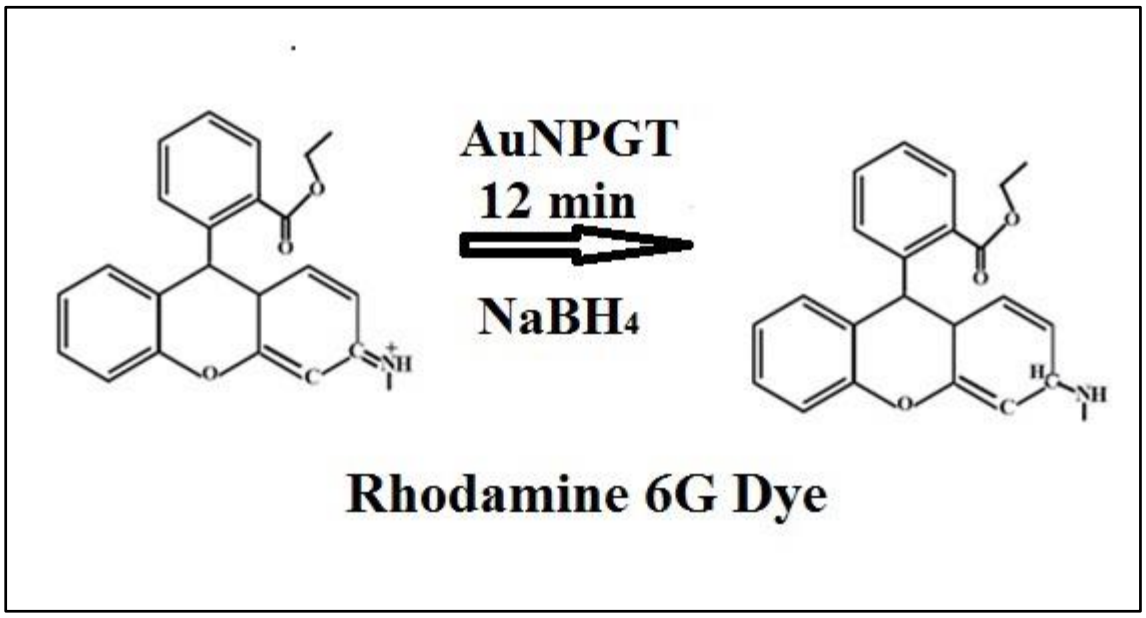


Figure 7. (a) UV -Visible spectra for the catalytic reduction of Rh6G. **(b)** The plots of ln C_0/C_t versus time for the reduction of Rh6G.



Scheme 1. The mechanism of catalytic reduction of Rh6G.

Free radical Scavenging activity of AuNPGT with DPPH

The free radical scavenging efficiency of the AuNPGT was represented in Figure 8 (a) which showed AuNPGT has significant antioxidant potential towards DPPH. Free radical % decreases with the amount of AuNPGT (10, 20, 30, 40, 50 µg/mL) increases (Amrutham et al. 2020), which may be due to that good scavenger can easily loss of electron. GT has showed not significant activity, maximum scavenging activity of AuNPGT was 70.9% at 50ug/mL and compares the standard AA. It has expressed value of IC_{50} = 31.56 µg/mL.

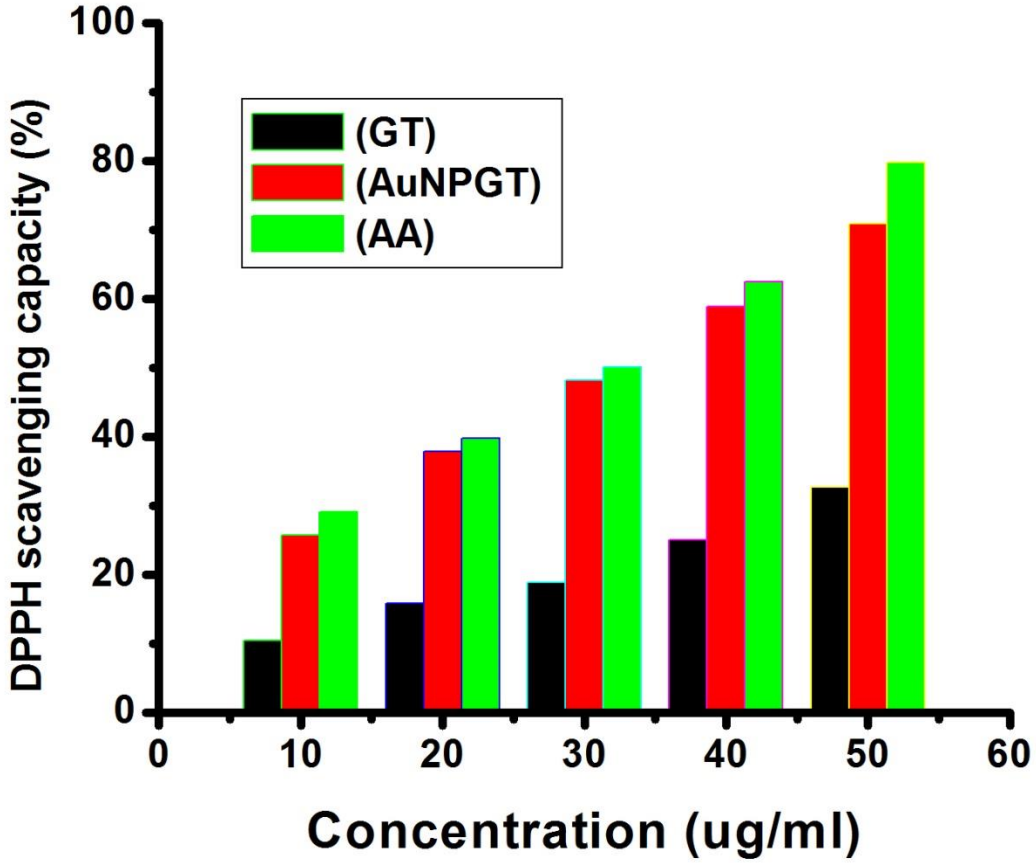


Figure 8. The free radical scavenging efficiency of the AuNPGT by DPPH

ABTS assay

The ABTS radical scavenging efficacy of the AuNPGT was showed in Figure 9 which showed significant scavenging potential towards ABTS. Absorbance calculated at 734 nm, scavenging activity increase with the amount of AuNPGT (10, 20, 30, 40, 50 µg/mL) increases. Maximum ABTS radical scavenging activity of AuNPGT was found to be 64.7 % at 50 µg/mL, control BHT 69.1.6% and the value observed concentration of IC_{50}= 28.2 µg/mL.

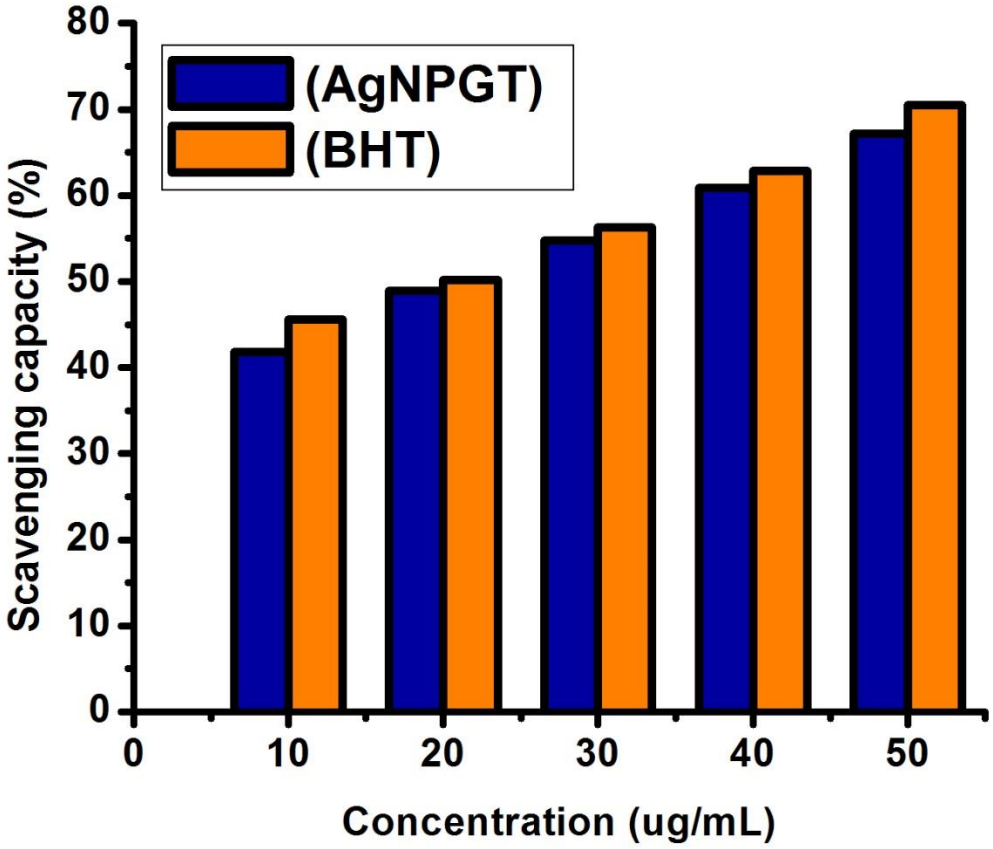


Figure 9. The free radical scavenging efficiency of the AuNPGT by ABTS.

Antimicrobial activity of AuNPGT

The effectiveness of AuNPGT was studied both gram positive and Gram negative bacteria correspondent S. aureus and E. coli. The exact mechanism which AuNPs are employed to cause an antimicrobial effect is not perfectly known and is an argued topic. However, several theories were written on the ability of AuNPs. It has the act like anchor to the bacterial cell wall and subsequently penetrates in to it and there by causing basic modification in the cell membrane like the permeability of the cell wall, consequence is loss of the cell. The cell wall is made up of phosphorus and sulfur, these are soft bases. The interaction of the AuNPs with the sulfur and phosphorus lead to replication in DNA of the bacteria and thus end the microbes (Slavin YN et al 2017). In the present study antibacterial studies of AuNPGT and GT alone were tested by the paper disc method using both S. aureus and E. coli are shown in Figure 10 shows zone of inhibition of AuNPGT and the pure GT has no inhibition ability.

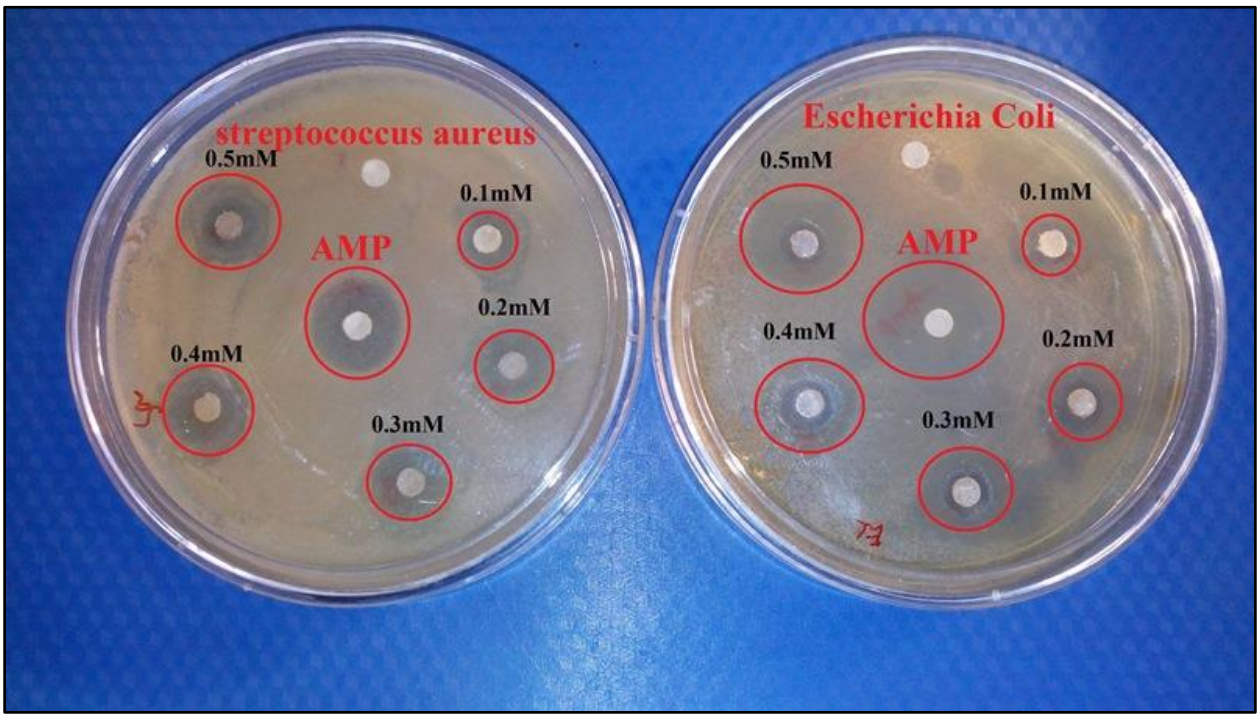


Figure 10. Antibacterial test results of AuNPGT with S. aureus and E. coli after 24 hours of incubation.

III. Conclusion

We have confirmed a microwave assisted green synthesis of AuNPGT with GT act as reducing agent and stabilizer. FTIR spectrum recognized hydroxyl groups responsible for the reducing the Au (III) to Au(0) state, which are present in GT. The investigations by UV–Vis- spectroscopy illustrate the formation of AuNPGT. The XRD and TEM measurements displayed that the resulting nanoparticles were faced centered cubic (fcc) structures and smaller than 12 nm in diameter. The green-synthesized AuNPGT was confirmed as efficient catalysts with enhanced rates of reduction of Rh6G dye reduction time was 12 min with rate constant 0.103287 min^{-1}. Radical scavenging activity performed against DPPH and ABTS with IC_{50} 31.56 and 28.2 µg/mL, The GT capped AuNPs showed significant antibacterial activity on both S.aureus and E.coli. Green synthesis of AuNPGT has proven that a promising material for catalytic mediator good scavenger and anti-bacterial agent.

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Compliance with Ethical Standards

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Research Involving Humans and Animals Statement None.

Informed Consent None.

Funding Statement None

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