

Green Synthesis of recyclable reduced graphene oxide-gold nanocatalyst using *Alstonia scholaris*: Applications in waste water purification and microbial field

Piu Das

The University of Burdwan

Bapan Bairy

The University of Burdwan

Sanjukta Ghosh

The University of Burdwan

Raktim Ghosh

The University of Burdwan

Somasri Dam

The University of Burdwan

Moni Baskey (Sen) (✉ moni.baskey@gmail.com)

University of Burdwan <https://orcid.org/0000-0003-2031-6929>

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Abstract

A novel green synthesis of reduced gold-graphene oxide (Au-RGO) nanocomposite was fabricated through simple heating method using *Alstonia scholaris* (*A. scholaris*) bark extract. The synthesized nanocatalyst was characterized by X-ray diffraction (XRD), UV-Visible spectroscopy, Fourier transformed infrared spectroscopy (FT-IR). Transmission electron microscopy (TEM) and size distribution study have been investigated to find the dimension of the gold nanoparticles (Au NPs) grown on reduced graphene oxide (RGO) surface. Subsequently, both the catalytic and photocatalytic performances of this nanocomposite were evaluated by using different organic hazardous dyes and a tentative photocatalytic reaction mechanism was discussed depending on the energy band position. To find the effect on different bacterial strains, antibacterial and antiprotozoal studies have been carried out with this nanocomposite.

Introduction

During the past few years, graphene is a kind of extensively explored and promoted nanomaterial among scientific researchers due to having unique two-dimensional, single-layer C – C sp²-bonded structure, excellent mechanical, thermal and electrical properties (Papageorgiou et al. 2017; Jung et al. 2017; Phiri et al. 2018; Liu et al. 2021). Besides, the high specific surface area of graphene makes it a perfect acceptor for nanoparticles (Wang et al. 2021; Zhang et al. 2018; lakunkov et al. 2019) and these engineered graphene-based two-dimensional carbon nanostructures serve as catalyst nanoparticles (Wang and Astruc 2018; Scheuermann et al. 2009; Li et al. 2010). By manipulating its interfacial property graphene or reduced graphene oxide is used as a material anchored with semiconductor and metal nanoparticles (Majumder and Gangopadhyay 2022; Kisieleska 2022; Kamat 2010). Until now, numerous hybrid nanocomposites have been constructed for different applications by assembling graphene with different nanomaterials. Meanwhile, the noble metal nanoparticles such as Ag, Pt, and Au have attracted much more attention in catalysis applications due to their unique physical and chemical properties (Arora et al. 2018).

Gold nanoparticles (Au NPs) are widely well known due to having excellent effects on optical and electronic, catalytic and photocatalytic properties (Bansal et al. 2020; Idisi et al. 2022; Murtaza et al. 2021; Perera et al. 2020). For these reasons, in scientific research Au NPs have superior applications such as electronics, photodynamic therapy, therapeutic agent delivery, sensors, probes, diagnostics, catalysis, imaging, etc. (Hu et al. 2020; Daraee et al. 2014; Amina et al. 2020). Moreover, functionalized gold nanomaterials with reduced graphene oxide (RGO) have been synthesized mostly in chemical ways. Such as, graphene-Au nanoparticles (Xu et al 2013; Goncalves et al. 2009) synthesized by chemical reduction method using particular reducing agents. Literature surveys describe that using reductive deposition, electrostatic self-assembly, microwave-assisted simultaneous reduction, wet impregnation thermal reduction, sonochemical reduction and self-catalysis reduction processes graphene-Au nanoparticles have been synthesized by Kong et al. 2009, Hu et al. 2012, Shervedani et al. 2014, Park et al. 2011 and Zhou et al. 2013 respectively. But, most of the proposed methods have several drawbacks including process complexity and cost ineffectiveness and they require the use of many chemicals, high-cost equipment and application of external energy. So, now scientists show their attention to the synthesis of graphene-based gold nanocomposites using plant extract which is a very cheap, cost-effective and easy growth step. There are several green synthesis procedures to synthesize Au-graphene nanocomposites, such as Willow-Leaved Knotgrass (El-Maghrabi et al. 2021), Xanthium Strumarium (Wen et al 2016), enzymatic glucose (Sadak et al. 2021), Syzygium cumini seed (Kadiyala et al. 2018), nisin peptides (Otari et al. 2017) etc.

A. scholaris is a species of traditional plant in the family Apocynaceae. It is commonly known as Saptaparni or Indian devil tree or in Bengali chatim. Extract of this tree contains indole, alkanoid and it has several usages in traditional medicine such as lung diseases, malaria, jaundice, gastrointestinal troubles, cancer, etc. (Chadha and Silakari 2017) in many parts of South-East Asia and the Indian subcontinent. To the best of our knowledge using *A. Scholaris* bark extract, the formation of Au-RGO nanocomposite has not been reported yet though synthesis of other nanomaterials has been studied (Table 1).

In our work, we have synthesized Au-RGO nanocomposite using *A. Scholaris* plant extract and this nanocomposite show efficient catalytic and photocatalytic performances to remove organic dyes. On the other side, the composite has good antimicrobial activity against Gram-positive bacteria like *B. subtilis* and *S. aureus* and the protozoan parasite *E. histolytica*.

Materials And Methods

Materials

The materials consists of ultrafine graphite powder (Aldrich), potassium permanganate (Merck), hydrogen peroxide (Merck, 30%), conc. sulfuric acid (Merck, 98% pure), hydrochloric acid (Merck, 30%), Gold (III) chloride (HAuCl₄) (Aldrich), *A. Scholaris* bark, liquor ammonia, 4-Nitroaniline (4-NA), sodium borohydride (NaBH₄), doubled distilled water, ethanol (Merck). All the above chemicals used here were of analytical grade reagents commercially available and used without further purification.

Preparation of extract

To prepare the bark extract, at first 20 g of bark of *A. Scholaris* was cleaned properly with doubled distilled water and then ground in smaller parts in a mortar pestle. The pasted bark was taken in a beaker and boiled for 1 hr after the addition of some water. After cooling the obtained transparent filtrate was used as a reducing agent after passing through a Whatman 41 filter paper to remove the solid materials.

Synthesis of reduced graphene oxide (RGO) and Au-RGO nanocomposites

Here, one of the precursors present in the reaction is graphene oxide (GO) nanosheet, which was synthesized from purified natural graphite by the modified Hummers method (William et al. 1958). The strategy for the synthesis of GO was described in our previous report (Tantubay et al. 2021) and reduced graphene oxide (RGO) was synthesized by using *A. Scholaris* bark extract as a reducing agent by the green method as illustrated in previously reported procedures (Ghosh et al. 2021). To synthesize Au-RGO nanocomposite, at first 0.21 g GO was dispersed in 100 mL doubled distilled water by ultra-sonication for 30 min to form a homogeneous dispersion and then 20 mL of 0.5 (μM) Gold (III) chloride solution was added to it under stirring condition. Then ammonia solution was added dropwise to the mixture to get the reaction medium alkaline. Thereafter, a known amount of bark extract (30 mL) obtained from *A. Scholaris* bark was added to the mixture. The resulting dispersion was heated at 60 °C for 2 hrs by continuous stirring on a magnetic stirrer. After completion of the precipitation reaction, the product was filtered and repeatedly washed with double distilled water and ethanol to remove any impurity adhered on the surface and finally dried in vacuum condition at 60 °C. The synthesis procedure of Au-RGO nanocomposite is shown in Scheme 1.

Characterization technique

The green synthesized RGO and Au-RGO nanocomposites were characterized using different spectroscopic and microscopic tools. Powder X-ray diffraction (XRD) measurement was carried out to analyze crystalline nature using a powdered sample with X-Ray powder Diffraction patterns, recorded in a Bruker (D8 Advance) diffractometer with Ni-filtered CuKα radiation ($\lambda = 1.5414 \text{ \AA}$). For phase identification recorded data was compared with standard JCPDS files (Joint Committee of Powder Diffraction System). To study the chemical bonding Fourier Transform Infrared (FTIR) study was performed by SHIMADZU IR Prestige-21 and the test specimens were prepared by the KBR disc method. The surface morphology of the as-synthesized RGO and Au-RGO nanocomposites was studied by transmission electron microscope (Model JEOL-1400) and to obtain the optical properties of the nanocatalysts, we used UV-Vis adsorption spectra by recording the data on SHIMADZU UV-1800 spectrophotometer.

Catalytic performance

Here, we report the catalytic properties of RGO and Au-RGO samples. In a typical catalysis reaction, the reduction of 4-NA to p-phenylene diamine (p-PDA) was studied in the presence of NaBH_4 . The catalytic reduction processes, Au-RGO, RGO, and without any catalyst (only 4NA+ NaBH_4) were carried out in a quartz cuvette and the reaction rate was monitored by successive UV-Vis absorbance measurements of the reaction solution as 4-NA and p-PDA exhibit distinct peaks at 380 and 238 nm, respectively. In a typical experiment, 6 mL of NaBH_4 (0.5 g in 20 mL) was added to 50.0 mL of 4-NA solution (0.1 mM). Then, 10.0 mg of each catalyst was added to the mixture. Aliquots of 5 mL volume were withdrawn at fixed intervals. After centrifugation, the concentration of the 4-NA was determined by measuring its absorbance in the wavelength range of 200–800 nm using the UV–Vis spectrophotometer. With the progress of the reaction, the light yellowish-colored 4-NA solution gradually changes to colorless with the formation of p-PDA which indicates the reduction properties for different samples. The percentage of dye degradation was estimated by the following formula:

$$\% \text{ of Dye degradation} = 100 \times (A_0 - A_t)/A_0 \dots\dots\dots (1)$$

Where A_0 is the initial absorbance of dye solution and A_t is the absorbance of dye solution after catalytic degradation at time 't'.

Photocatalytic Performance

To show the catalytic activity of the Au-RGO nanocomposite and RGO sample in presence of a photon, photocatalytic degradation studies were performed by different industrial cationic and anionic dyes such as methylene blue, methyl orange. To carry out the photocatalytic performance 10 mg of the sample was added to 50 mL of two different dye solutions (2.5 mg L^{-1}) separately and the mixture was stirred for 30 min in a dark environment to attain adsorption–desorption equilibrium. After 30 min of adsorption–desorption process, the visible light 500-W xenon lamp was irradiated over the solution with constant stirring. UV–Vis absorption data were taken continuously after certain time intervals and the absorbance was noted using the UV-visible spectrometer. Measuring the absorbance intensity during the photocatalytic degradation process, % of Degradation was calculated using the relation in Equation (1).

Antibacterial activity

The antibacterial activity of Au-RGO nanocomposite was tested against bacteria strains *Bacillus subtilis* (Gram-positive), *Escherichia coli* BL21 (DE3) (Gram-negative), and *Staphylococcus aureus* (Gram-positive) by well diffusion method (Das et al. 2018). Mueller Hinton Agar medium (HiMedia, India) was used in this study. 100 μl of inoculums from log-phase grown cells were spread separately on the solid surface of the plates containing Mueller Hinton Agar medium. Using a sterile cork borer the wells of 3 mm in diameter were made. 50 μl of sample solution of both Au-RGO and RGO were placed in the wells and incubated at 37 °C for 24 hours in a bacterial incubator. The zone of inhibition that appeared in the surrounding the wells was noted and compared against standard antibiotics ampicillin and tetracyclin. The experiments were done in triplicates.

Antiprotozoal activity

The antiprotozoal activity of Au-RGO was tested against the human pathogen *Entamoeba histolytica*, a protozoan parasite. *E. histolytica* trophozoites of strain HM-1: IMSS were cultured axenically using TYI-S-33 medium, supplemented with 10% adult bovine serum, antibiotic solution of 100 U/ml penicillin, and 0.25 mg/ml streptomycin (Himedia, India). Trophozoites were grown in Nunc™ cell culture tubes (Thermo Fisher Scientific, USA) at 37°C phase, tubes were chilled on ice for 20 minutes to detach the cells and centrifuged at 500 g for 5 minutes. The supernatant was removed, and the cells were re-suspended in a fresh medium. A fresh culture of 11×10^3 trophozoites per milliliter was introduced in each well of a 48-well tissue culture plate (Tarsons, India). After the attachment of trophozoites at the bottom of the wells, sample solutions were placed in the wells and incubated at 37 °C for 24 hours and 48 hours in an incubator. Trophozoites were counted using a hemocytometer under an inverted microscope (Zeiss, Germany). Three experiments were performed at each time point separately.

Results And Discussions

To characterize the nanocomposite of RGO, the XRD patterns have been carried out of that sample along with GO and RGO shown as shown in Fig. 1a. The peak at $2\theta = 13.54^\circ$ for GO (Huang et al. 2018) and peaks at $2\theta = 24.56^\circ$ and 42.58° in case of RGO (Abarca et al. 2020) confirms the formation of GO and RGO respectively. The high-intensity peaks of Au-RGO nanocomposite at the $2\theta =$ values of 38.14, 44.28, 64.47, and 77.54 correspond to (111), (200), (220) and (311) planes respectively of Au nanoparticles grown on RGO surface which were compared with standards JCPDS: 004-078489-3697 data (Fatemeh et al. 2021). With the formation of Au nanoparticles, the peak due to the (002) plane was right shifted to 31.7° 2θ value. This is because of the fact that due to the generation of internal stress for the doping and crystal defects (Ruidáz-Martínez et al. 2020) during the formation of Au-RGO nanocomposite.

To explore the changes in chemical composition and characterize the functional groups present in the sample, the Fourier transform infrared spectroscopy (FTIR) was carried out for the samples GO, RGO and Au-RGO nanocomposite Fig. 1b. FTIR spectrum of GO peak $3361, 1695 \text{ cm}^{-1}$ are attributed to O–H stretching and bending vibration (Rattana et al. 2012) respectively and 2343 cm^{-1} is for asymmetric stretching of C = O bond in carboxyl group (Gong et al. 2015) situated at the edge of GO sheet respectively. The band at $1070\text{--}1200 \text{ cm}^{-1}$ is responsible for epoxy C-O bond (Emiru and Ayele 2017) which is almost disappeared in case of Au-RGO nanocomposite which indicates the successful formation of RGO nanocomposite (Sivalingam and Balasubramanian 2017).

UV-Visible spectrum in Fig. 2a, GO exhibited an absorption peak centered at 231 nm which is attributed to $\pi\text{--}\pi^*$ transition of aromatic C = C bond present in GO layers and 310 nm, originated due to the presence of $n\text{--}\pi^*$ transition of carboxyl and other carbonyl and hydroxyl auxochromes (Das et al. 2018; Amouzadeh et al. 2014). In the RGO sample, the peak responsible for $n\text{--}\pi^*$ transitions disappears and a new peak is shown at 270 nm (Amanulla et al. 2017) indicating the reduction of GO to RGO. On the reduction band at 231 nm red-shifted to 264 nm and the band at 310 nm disappear. In the composite sample presence of a peak at 264 nm and a hump between 500–600 nm (Santhoshkumar et al. 2017) indicates the formation of Au-RGO nanocomposite.

The optical band gap study has been investigated from the Tauc plot using the following equation (Makula et al. 2018):

$$(\alpha h\nu)^n = K(h\nu - E_g)$$

2

Where, $h\nu$ is the photon energy, α is the absorption coefficient, K is a constant, E_g is the band gap energy and the value of n is 2 for direct allowed transitions while it is $\frac{1}{2}$ for the indirect allowed transition. The plot of $(\alpha h\nu)^2$ vs. $h\nu$ (Fig. 2b,c,d) gives the band gap value by extrapolating the linear portion to the $h\nu$ axis. The obtained band gap values are 3.34, 1.88 and 2.91 eV for GO, RGO and Au-RGO nanocomposite respectively. This is because of the fact that, after a reduction from GO to RGO band gap decreases due to the cancellation of oxygenated groups (Jin et al. 2020). The increased band gap in nanocomposite than that of RGO is due to the formation of functionalized RGO (Al-Hossainy et al. 2015).

Transmission electron microscopy (TEM) has been carried out to identify the shape, and size of the as-prepared RGO sample and Au-RGO nanocomposite synthesized by the green method. The TEM image of RGO shows like few layers of nanosheets (Fig S1) and it can be revealed that the predominantly spherical Au NPs are uniformly anchored on the surface of RGO as shown in Fig. 3a. The dimension of the maximum number of NPs is 5–10 nm which is clear from the particle distribution graph Fig. 3b. The average diameter of Au NPs (8 nm) in Au-RGO composite is in good agreement with the XRD result.

Catalytic activity study

Catalytic reduction

The catalytic performance of the green RGO and Au-RGO nanocomposite has been investigated in presence of NaBH_4 by using the reduction of 4-NA into p-PDA. To compare the catalytic efficiency of the nanocomposite, we performed the same reduction reaction without adding any catalyst and the results were depicted in Table 2. The produced p-PDA as a reduction product of 4-NA (Wu et al. 2012) is considered a useful and attractive intermediate in the preparation of different polymers, hair dyes, and rubber products. The light yellowish solution of 4-NA exhibited a strong absorption

peak at 380 nm (Zheng et al. 2015) and with the progress of reduction, the solution gradually turns colorless, the peak intensity decreases and a new peak at about 240 nm appears with the formation of p-PDA (Li et al. 2018). It was shown that when only RGO is used as a catalyst, the intensity of absorbance peak decreases very slowly within a fixed time i.e within 63 min, approximately 34.5 % reduction reaction occurs. But after the addition of Au-RGO nanocomposite into the system the formation of p-PDA has been completed rapidly with the conversion exceeding 94.4 % at a reaction time of about 63 min [Fig. 4a-e]. But in absence of RGO and Au-RGO catalyst i.e in presence of only NaBH₄, approximately 12.9 % reduction of 4-NA occurred in same time intervals.

Table 2 Comparison of the degradation efficiency, the rate constant and relative correlation coefficient of catalytic

Reduction

Sample	% of Degradation	Rate Constant (Min ⁻¹)	R ² value
Without Catalyst	12.9%	0.00133	0.00528
RGO	34.5%	0.00528	0.75408
Au-RGO	94.4%	0.04451	0.9988

To study the kinetic phenomena of catalytic reduction, the reaction has been investigated by the Eq. (3),

$$\ln \frac{A_0}{A_t} = kt.....(3)$$

where, A₀ = initial absorbance, A_t = absorbance at time t, k=rate constant. The catalytic reaction is calculated from A₀/A_t measured at 380 nm. Herein, As shown in Fig. 4e, the linear correlation between ln (A₀/A_t) and reaction time demonstrates that the reaction follows a pseudo-first-order reaction and the obtained values of rate constant for without catalyst, RGO and Au-RGO are listed in Table 2. It is concluded that the catalytic efficiency for the reduction of 4-NA is approximately 33.5 and 3.97 times enhanced in case of Au-RGO nanocomposite and RGO respectively. The excellent catalytic activity of Au-RGO nanocomposite could be attributed due to highly dispersed small-sized Au NPs spread on RGO sheet.

The plausible reduction mechanism of 4-NA at the surface of Au NPs grown on RGO sheet is shown schematically in scheme 2. When NaBH₄ is added in aqueous solution, BH₄⁻ is produced and due to having strong nucleophilic behavior, BH₄⁻ gives electron to catalyst and produce hydride ions. The hydride ions then react with proton (H⁺) supplied by water to generate hydrogen molecules (H₂) and then get adsorbed at the surface of Au NPs grown on RGO sheet by Vander Waals attractive forces. This adsorbed hydrogen then attack at the nitro group of 4-NA and reduces it to p-PD A. When 4-NA is reduced at the surface of Au-RGO nanocomposite, the formed product is desorbed from the surface of the nanocomposite and in this way surface of sample is regenerated for further step.

Reusability test

It is well-known that the stability of materials has a very important influence on their application areas. So, to investigate the stability of Au-RGO nanocatalyst reusability test was carried out by performing five subsequent 4-NA/NaBH₄ reactions by recovering the catalyst from each cycle of the reaction as shown in Fig.4f. For all the five cycles the reagents, amount of catalyst and the reaction times have been kept unchanged. The percentage of reducing ability of the 4NA dye in a fixed time and it was observed that the catalyst has good reusability up to 5 cycles.

Photocatalytic study

The photocatalytic study has been investigated with the Au-RGO nanocomposite sample on the degradation of MB and MO dyes. Actually, when a semiconductor absorbs a photon of equal energy or larger than the semiconductor band gap, electron-hole pair is generated. The electron is excited to conductance band and at the same time the hole

is created in the valence band as shown in Scheme 3. Greater the charge separation lesser will be the probability of recombination and thus photocatalytic efficiency will be increased. With the decrease of particle size of Au NPs band gap increases as a result photocatalytic ability increases. At first excited electron is trapped by the RGO surface and after moving through the surface that electron reduces the dissolved oxygen to peroxide radicals and the hole in the valence band oxidizes the water to hydroxyl radicals. These radicals (O₂⁻, OH[·]) are highly responsible for converting the organic dye into degraded product. Thus due to well charge separation photocatalytic ability enhances. From the Fig. 5(a-c), it is shown that in presence of photon the catalytic efficiency of Au-RGO nanocomposite is greater in case of MB than that of MO. This is because of the fact that MB is attached with RGO sheet with both π-π and electrostatic interactions but in case of MO only π-π interaction works. Therefore greater the attachment of dye molecule with the RGO sheet greater will be the photocatalytic efficiency. The rate kinetics (Fig. 5d) has been investigated in case of both dyes using Equation 3 and the results of photocatalytic efficiency of the nanocomposite have been tabulated in Table 3.

Table 3 Comparison of the degradation efficiencies, the rate constants and relative correlation coefficients of MB and MO

Dye Name	% of Degradation	Rate Constant (Min ⁻¹)	R ² value
Methylene Blue (MB)	98%	0.0222	0.97909
Methylene Orange (MO)	14%	0.000569	0.94576

Antibacterial activity and antiprotozoal study

Antibacterial activity of nanoparticles shows that Au-RGO has an inhibitory effect on the growth of some Gram-positive bacteria like *B. subtilis* and *S. aureus*, but no significant effect has been seen in *E. coli* (Fig. 6 and Table 4b). The reason might be recognized to the presence of an additional outer membrane which may reduce the penetration rate of Au-RGO through the membrane. In this study, no antibacterial effect of RGO has been observed in this concentration (200 µg mL⁻¹). The antiprotozoal activity of Au-RGO and RGO are summarized in Table 5. It has been seen that the number of *E. histolytica* trophozoites decreased after 24 and 48 hours of incubation in the Au-RGO treated tubes, compared to the control. Only RGO shows a very low inhibitory effect on the growth of trophozoites. The reduction of trophozoites growth after Au-RGO treatment may happen due to the production of reactive oxygen species (ROS) that may induce oxidative stress and cytotoxic effect in cells (Mateo et al. 2014; Enea et al. 2020). Though *E. histolytica* has its own antioxidant strategy to protect itself against reactive oxygen species, most of the antioxidant defense mechanisms such as glutathione peroxidase, glutathione reductase, and catalase are not present in this parasite (Chakraborti et al. 2019). Therefore it is clear from the study, that the gold nanoparticle Au-RGO has significant activity against the protozoan parasite *E. histolytica* and other Gram-positive bacteria.

Table 4a Antibacterial activity of standard antibiotics against bacterial strains

Bacterial strain	Concentration of antibiotics (mg mL ⁻¹)	Zone of inhibition for antibiotics (mm in diameter)		Table 4b Antibacterial activity of synthesized nanomaterials
		Ampicillin	Tetracyclin	
<i>Escherichia coli</i>	10	23.34±0.58	31.34±0.58	Table 5 Antiprotozoal activity of nanomaterials
<i>Bacillus subtilis</i>	10	21.67±0.58	21.34±0.58	
<i>Staphylococcus aureus</i>	10	32±1	14.33±0.58	

Nanocomposite	Bacterial strain	Concentration of nanocomposite (µg mL ⁻¹)	Volume of nanocomposite (µL)	Zone of inhibition for nanocomposite (mm in diameter)
Au-RGO	<i>Escherichia coli</i>	200	50	-
	<i>Bacillus subtilis</i>	200	50	10±0.5
	<i>Staphylococcus aureus</i>	200	50	9.67±0.58
RGO	<i>Escherichia coli</i>	200	50	-
	<i>Bacillus subtilis</i>	200	50	-
	<i>Staphylococcus aureus</i>	200	50	-

Cell culture of <i>E. histolytica</i>	Conc ⁿ of nanocomposite Au-RGO (µg mL ⁻¹)	Volume of nanocomposite Au-RGO (µL)	Cell count after 24 hours of incubation (cells mL ⁻¹)		Cell count after 48 hours of incubation (cells mL ⁻¹)	
			Au-RGO	Control	Au-RGO	Control
Initial cell volume- 11×10 ³ cells mL ⁻¹	200	10	8.67×10 ³ ±577.3503	15.34×10 ³ ±577.3503	5.67×10 ³ ±577.3503	21.67×10 ³ ±577.3503

Cell culture of <i>E. histolytica</i>	Conc ⁿ of nanoparticle RGO (µg mL ⁻¹)	Volume of nanoparticle RGO (µL)	Cell count after 24 hours of incubation (cells mL ⁻¹)		Cell count after 48 hours of incubation (cells mL ⁻¹)	
			RGO	Control	RGO	Control
Initial cell volume- 11×10 ³ cells mL ⁻¹	200	10	14.34×10 ³ ±577.3503	15.34×10 ³ ±577.3503	19.67×10 ³ ±577.3503	21.67×10 ³ ±577.3503

Conclusions

In this chapter, we can conclude that, Au-RGO nanocomposite was successfully synthesized using green approach i.e., using *A. Scholaris* bark plant extract. The NPs formed on RGO surface were observed to be spherical in shape and the average dimension of the Au NPs is 5–10 nm. The particle distribution graph also supports the result of TEM study. The prepared nanocomposite was evaluated for their excellent catalytic activity using 4NP dye degradation and also showed superior photocatalytic performances for degradation of methylene blue dye. So, this nanocomposite may be used as remarkable nanocatalysts for wastewater management. The Au-RGO nanocomposite was also found to be selectively effective in various strains of microorganisms and show potential activity as antibacterial and antiprotozoal agents.

Declarations

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Competing Interests

There are no competing interests.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to Participate

All participants voluntarily agreed to participate in this research study.

Consent to Publish

All participants voluntarily agreed to publish their research work in Springer journal.

Authors Contributions

PD contributed to writing—original draft, writing—review and editing, conceptualization, methodology,

investigation. SG contributed to conceptualization, data curation, visualization. BB contributed to data curation and formal analysis. RG contributed to methodology and validation. SD contributed to formal analysis and investigation. MBS contributed to supervision, writing and conceptualization.

Availability of data and materials

The data that support the findings of this study are available from authors.

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References

1. Kozłowski W, Piwoński I (2022) The role of graphene oxide and its reduced form in the in situ photocatalytic growth of silver nanoparticles on graphene-TiO₂ nanocomposites. *Appl Surf Sci* 576: 151759. <https://doi.org/10.1016/j.apsusc.2021.151759>.
2. Kong BS, Geng J, Jung HT (2009) Layer-by-layer assembly of graphene and gold nanoparticles by vacuum filtration and spontaneous reduction of gold ions. *Chem Commun* 16: 2174–2176. <https://doi.org/10.1039/B821920F>.
3. Li Q, Xia Y, Yang C, Lv K, Lei M, Li M (2018) Building a direct Z-scheme heterojunction photocatalyst by ZnIn₂S₄ nanosheets and TiO₂ hollowspheres for highly-efficient artificial photosynthesis. *Chem Eng J* 349: 287–296. <https://doi.org/10.1016/j.cej.2018.05.094>.
4. Li Y, Fan X, Qi J, Ji J, Wang S, Zhang G, Zhang F (2010) Gold nanoparticles–graphene hybrids as active catalysts for Suzuki reaction. *Mater Res Bull* 45(10):1413–1418. <https://doi.org/10.1016/j.materresbull.2010.06.041>.
5. Liu Y, Zheng J, Zhang X, Li K, Du Y, Yu G, Jia Y, Zhang Y (2021) Recent advances on graphene microstructure engineering for propellant-related applications. *J Appl Polym Sci* 1-22. <https://doi.org/10.1002/app.50474>.
6. Majumder P, Gangopadhyay R (2022) Evolution of graphene oxide (GO)-based nanohybrid materials with diverse compositions: an overview. *RSC Adv* 12:5686-5719. <https://doi.org/10.1039/D1RA06731A>.
7. Makuła P, Pacia M, Macyk W (2018) How to correctly determine the band gap energy of modified semiconductor photocatalysts based on UV–Vis spectra. *J Phys Chem Lett* 9(23): 6814–6817. <https://doi.org/10.1021/acs.jpclett.8b02892>.
8. Mateo D, Morales P, Ávalos A, Haza AI (2014) Oxidative stress contributes to gold nanoparticle-induced cytotoxicity in human tumor cells. *Toxicol Mech Methods* 24(3): 161–172. <http://dx.doi.org/10.3109/15376516.2013.869783>.
9. Murtaza A, Uroos M, Sultan M, Muazzam R, Naz S (2021) Enhancing catalytic potential of gold nanoparticles by linear and cross-linked polyurethane blending. *RSC Adv* 11: 26635-26643. <https://doi.org/10.1039/D1RA04662D>.
10. Otari SV, Kumar M, Anwar MZ, Thorat ND, Patel SKS, Lee D, Lee JH, Lee JK, Kang YC, Zhang L (2017) Rapid synthesis and decoration of reduced graphene oxide with gold nanoparticles by thermostable peptides for memory device and photothermal applications. *Sci Rep* 7(1): 10980–10994. <https://doi.org/10.1038/s41598-017-10777-1>.
11. Papageorgiou DG, Kinloch IA, Young RJ (2017) Mechanical properties of graphene and graphene-based nanocomposites. *Prog Mater Sci* 90:75-127. <https://doi.org/10.1016/j.pmatsci.2017.07.004>.
12. Park G, Lee KG, Lee SJ, Park TJ, Wi R, Kim DH (2011) Synthesis of graphene-Gold nanocomposites via sonochemical reduction. *J Nanosci Nanotechnol* 11(7): 6095–6101. <https://doi.org/10.1166/jnn.2011.4446>.
13. Perera M, Wijanayaka LA, Siriwardana K, Dahanayake D, Nalin de Silva KM. (2020) Gold nanoparticle decorated titania for sustainable environmental remediation: green synthesis, enhanced surface adsorption and synergistic photocatalysis. *RSC Adv* 10:29594-29602. <https://doi.org/10.1039/D0RA05607C>.
14. Phiri J, Johansson LS, Gane P, Maloney T (2018) A comparative study of mechanical, thermal and electrical properties of graphene, graphene oxide- and reduced graphene oxide-doped microfibrillated cellulose nanocomposites. *Compos B Eng* 147: 104-113. <https://doi.org/10.1016/j.compositesb.2018.04.018>.
15. Rajasekar R, Samuel M, Edison TNJI, Raman N (2021) Sustainable synthesis of silver nanoparticles using *Alstonia scholaris* for enhanced catalytic degradation of methylene blue. *J Mol Struct* 1246: 131208-131214. <https://doi.org/10.1016/j.molstruc.2021.131208>.
16. Rattana, Chaiyakun S, Witit-anun N, Nuntawong N, Chindaudom P, Oaew S, Kedkeaw C, Limsuwan P (2012). Preparation and characterization of graphene oxide nanosheets. *Procedia Eng* 32: 759–764. <https://doi.org/10.1016/j.proeng.2012.02.009>.
17. Ruid  az-Mart    ez M,   lvarez MA, L    pez-Ram    n MV, Cruz-Quesada G, Rivera-Utrilla J, S    nchez-Polo M (2020). Hydrothermal Synthesis of rGO-TiO₂ Composites as High-Performance UV Photocatalysts for Ethylparaben Degradation. *Catalysts* 10(5):520–545. <https://doi.org/10.3390/catal10050520>.

18. Sadak O (2021) One-pot scalable synthesis of rGO/AuNPs nanocomposite and its application in enzymatic glucose biosensor. *Nanocomposites* 7(1):44-52. <https://doi.org/10.1080/20550324.2021.1917837>.
19. Santhoshkumar J, Rajeshkumar S, Venkat Kumar S. (2017) Phyto-assisted synthesis, characterization and applications of gold nanoparticles – A review. *Biochem Biophys Rep* 11: 46–57. <https://doi.org/10.1016/j.bbrep.2017.06.004>.
20. Scheuermann GM, Rumi L, Steurer P, Bannwarth W, Mülhaupt R (2009) Palladium nanoparticles on graphite oxide and its functionalized graphene derivatives as highly active catalysts for the suzuki–miyaura coupling reaction. *J Am Chem Soc* 131(23):8262–8270. <https://doi.org/10.1021/ja901105a>.
21. Shahid S, Ejaz A, Javed M, Mansoor S, Iqbal S, Elkaeed EB, Alzhrani RM, Alsaab HO, Awwad NS, Ibrahim HA, Fatima U, Zaman S, Sarwar MN (2022) The Anti-Inflammatory and Free Radical Scavenging Activities of Bio-Inspired Nano Magnesium Oxide. *Front Mater* 9:875163. <https://doi.org/10.3389/fmats.2022.875163>.
22. Sherdedani RK, Amini A (2014) Novel graphene-gold hybrid nanostructures constructed via sulfur modified graphene: Preparation and characterization by surface and electrochemical techniques. *Electrochim Acta* 121: 376–385. <https://doi.org/10.1016/j.electacta.2013.12.044>.
23. Shimpi NG, Shirole S, Mishra S (2015) Biogenesis synthesis and characterization of silver nanoparticles (AgNPs) using the aqueous extract of *Alstonia scholaris*: A greener approach. *Micro Nanosyst* 7: 49-54. <http://dx.doi.org/10.2174/1876402907666150609224649>.
24. Sivalingam MM, Balasubramanian K, (2017). Influence of the concentration of reducing agent on gold nanoparticles decorated reduced graphene oxide and its ammonia sensing performance. *Appl Phys A* 123(4):281. <https://link.springer.com/article/10.1007%2Fs00339-017-0910-9>.
25. Supraja N, Prasad TNKV, Gandh AD, Anbumani D (2018) Synthesis, characterization and evaluation of antimicrobial efficacy and brine shrimp lethality assay of *Alstonia scholaris* stem bark extract mediated ZnONPs. *Biochem Biophys Rep* 14:69-77. <https://doi.org/10.1016/j.bbrep.2018.04.004>.
26. Supraja N, Tollamadugu NVKVP, Adam S (2016) Phytogenic silver nanoparticles (*Alstonia scholaris*) incorporated with epoxy coating on PVC materials and their biofilm degradation studies. *Adv Nano Res* 4(4): 281-294. <http://dx.doi.org/10.12989/anr.2016.4.4.281>.
27. Tantubay K, Das P, Baskey Sen M, (2021) Hydrogen peroxide–assisted photocatalytic dye degradation over reduced graphene oxide integrated ZnCr₂O₄ nanoparticles. *Environ Sci Pollut Res* 29: 148–155. <https://doi.org/10.1007/s11356-021-17105-1>.
28. Tripathi SK, Mahakud AK, Biswal BK (2019) A green approach towards formulation, characterization, and antimicrobial activity of Poly (Lactic-co-Glycolic) Acid-*Alstonia scholaris* based nanoparticles. *Mater Res Express* 1-21. <https://doi.org/10.1088/2053-1591/ab30d1>.
29. Wang C, Astruc D (2018) Recent developments of metallic nanoparticle-graphene nanocatalysts. *Prog Mater Sci* 94:306-383. <https://doi.org/10.1016/j.pmatsci.2018.01.003>.
30. Wang K, Hui KN, Hui KS, Peng S, Xu Y (2021) Recent progress in metal–organic framework/graphene-derived materials for energy storage and conversion: design, preparation, and application. *Chem Sci* 12: 5737-5766. <https://doi.org/10.1039/D1SC00095K>.
31. Wen J, Salunke BK, Kim BS (2016) Biosynthesis of graphene-metal nanocomposites using plant extract and their biological activities. *J Chem Technol* 92(6):1-27. <https://doi.org/10.1002/jctb.5140>.
32. William S, Hummers J, Offeman RE (1958) Preparation of graphitic oxide. *J Am Chem Soc* 80:1339. <https://doi.org/10.1021/ja01539a017>.
33. Wu W, Liu G, Xie Q, Liang S, Zheng H, Yuan R, Su W, Wu L (2012) A simple and highly efficient route for the preparation of p-phenylenediamine by reducing 4-nitroaniline over commercial CdS visible light-driven photocatalyst in water. *Green Chem* 14(6): 1705–1709. <https://doi.org/10.1039/C2GC35231A>.
34. Xu M, Wu JF, Zhao GC (2013) Direct electrochemistry of hemoglobin at a graphene gold nanoparticle composite film for nitric oxide biosensing. *Sensors* 13(6):7492–7504. <http://dx.doi.org/10.3390/s130607492>.
35. Zhang H, Guo H, Li A, Chang X, Liu S, Liu D, Wang Y, Zhang F, Yuan H (2018) High specific surface area porous graphene grids carbon as anode materials for sodium ion batteries. *J Energy Chem* 31:159-166. <https://doi.org/10.1016/j.jechem.2018.06.002>.
36. Zheng K, Zhang T, Lin P, Han Y, Li H, Ji R, Zhang H (2015). 4-Nitroaniline degradation by TiO₂ catalyst doping with manganese. *J Chem* 2015: 1–6. <https://doi.org/10.1155/2015/382376>.
37. Zhuo Q, Ma Y, Gao J, Zhang P, Xia Y, Tian Y, Sun X, Zhong J, Sun X (2013) Facile synthesis of graphene/metal nanoparticle composites via self-catalysis reduction at room temperature. *Inorg Chem* 52(6): 3141–3147. <https://doi.org/10.1021/ic302608g>.

Table 1

Table 1 is available in the Supplementary Files section.

Schemes

Scheme 1 to 3 are available in the Supplementary Files section.

Figures

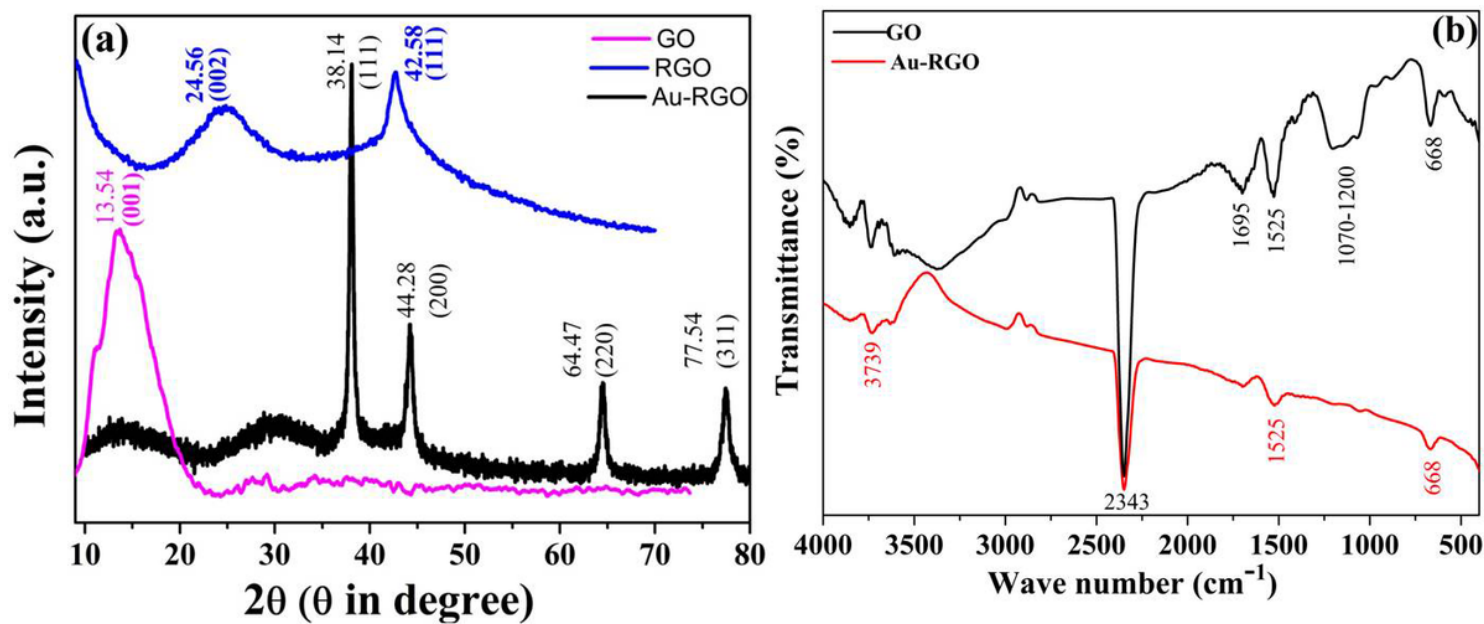


Figure 1

a XRD graphs of GO, RGO, Au-RGO and b FTIR spectra of GO and Au-RGO

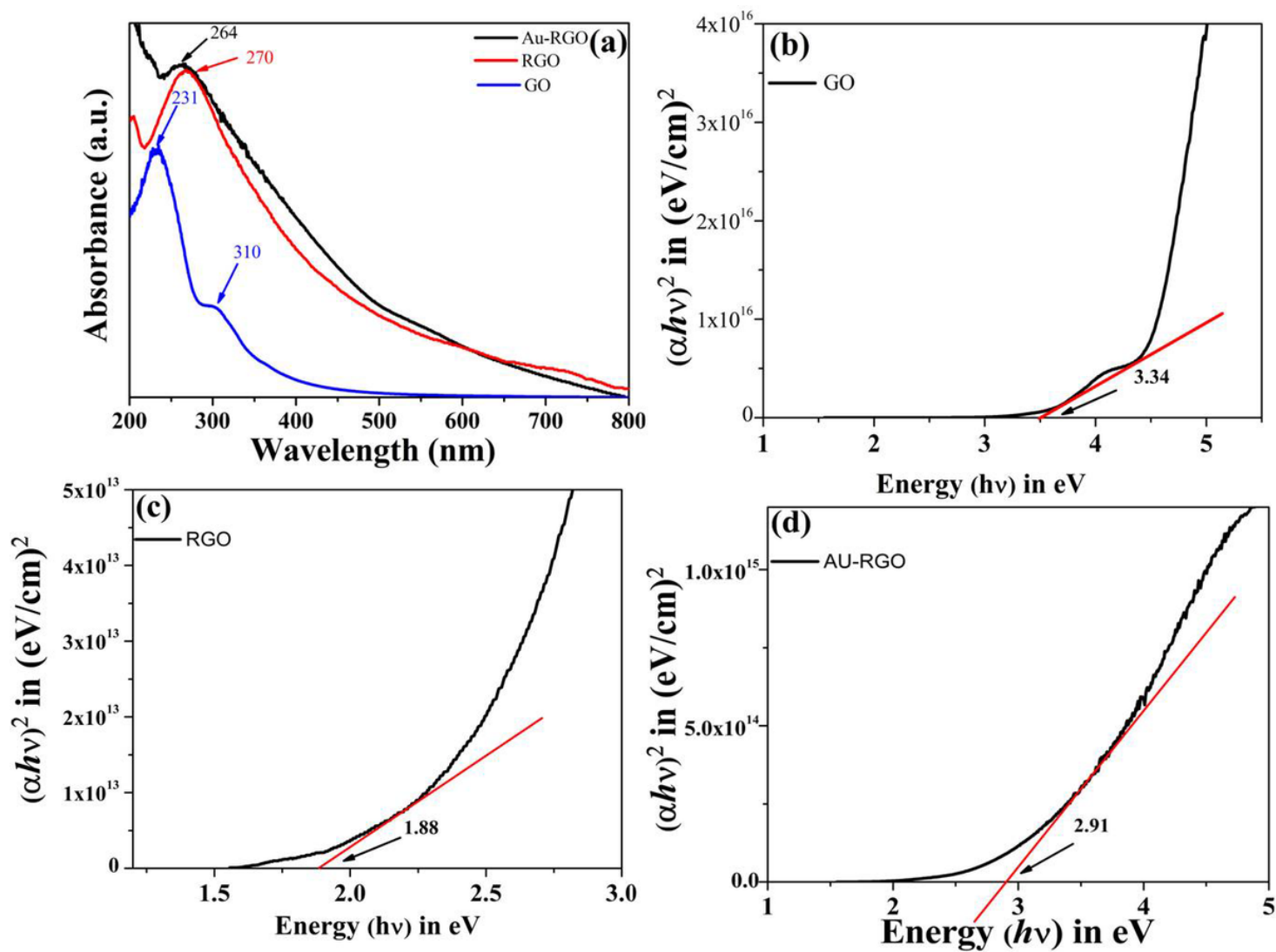


Figure 2

a UV-Vis spectra of a GO, RGO and Au-RGO nanocomposites and UV-Vis absorption data fitted by Tauc's formula for direct band gap of b GO, c RGO, and d Au-RGO nanocomposites

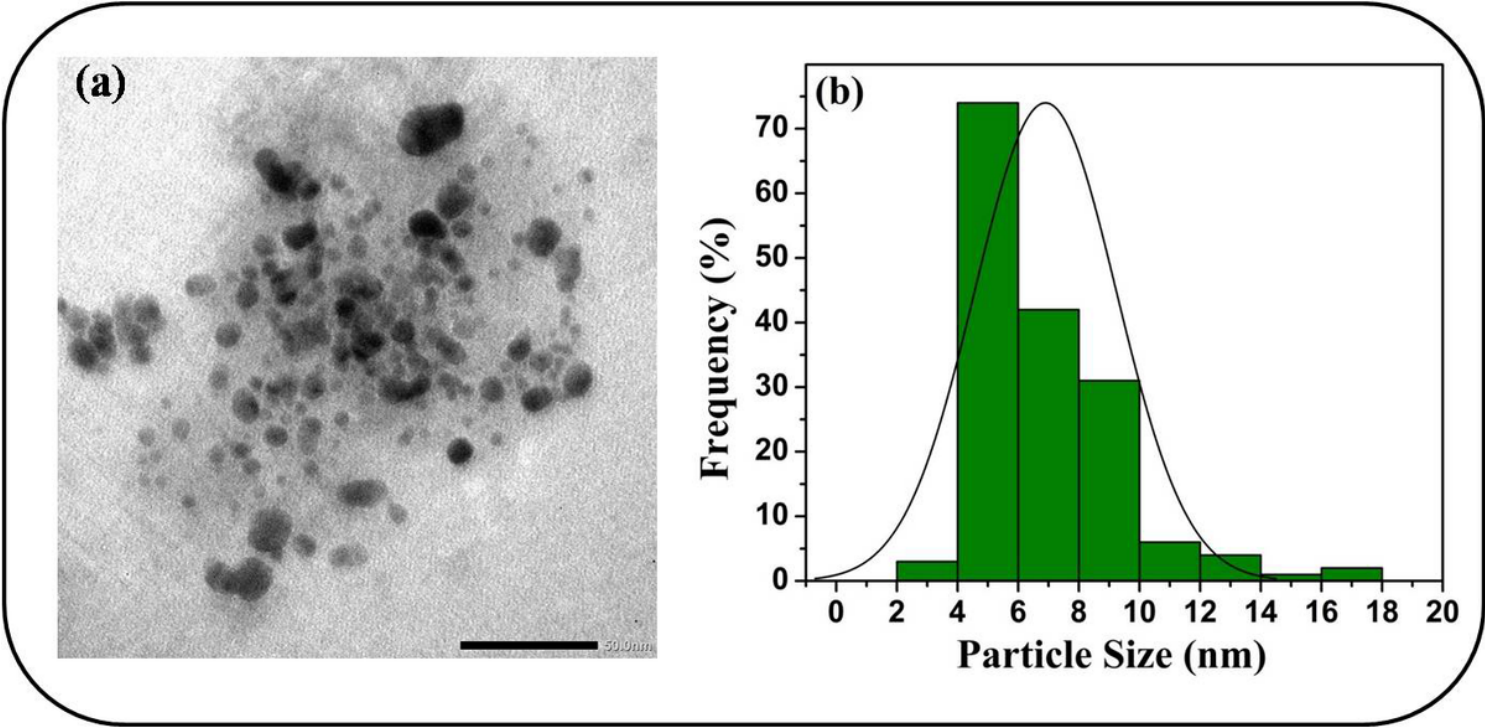


Figure 3

TEM images of a Au-RGO nanocomposites and b size distribution graph of Au-RGO nanocomposites

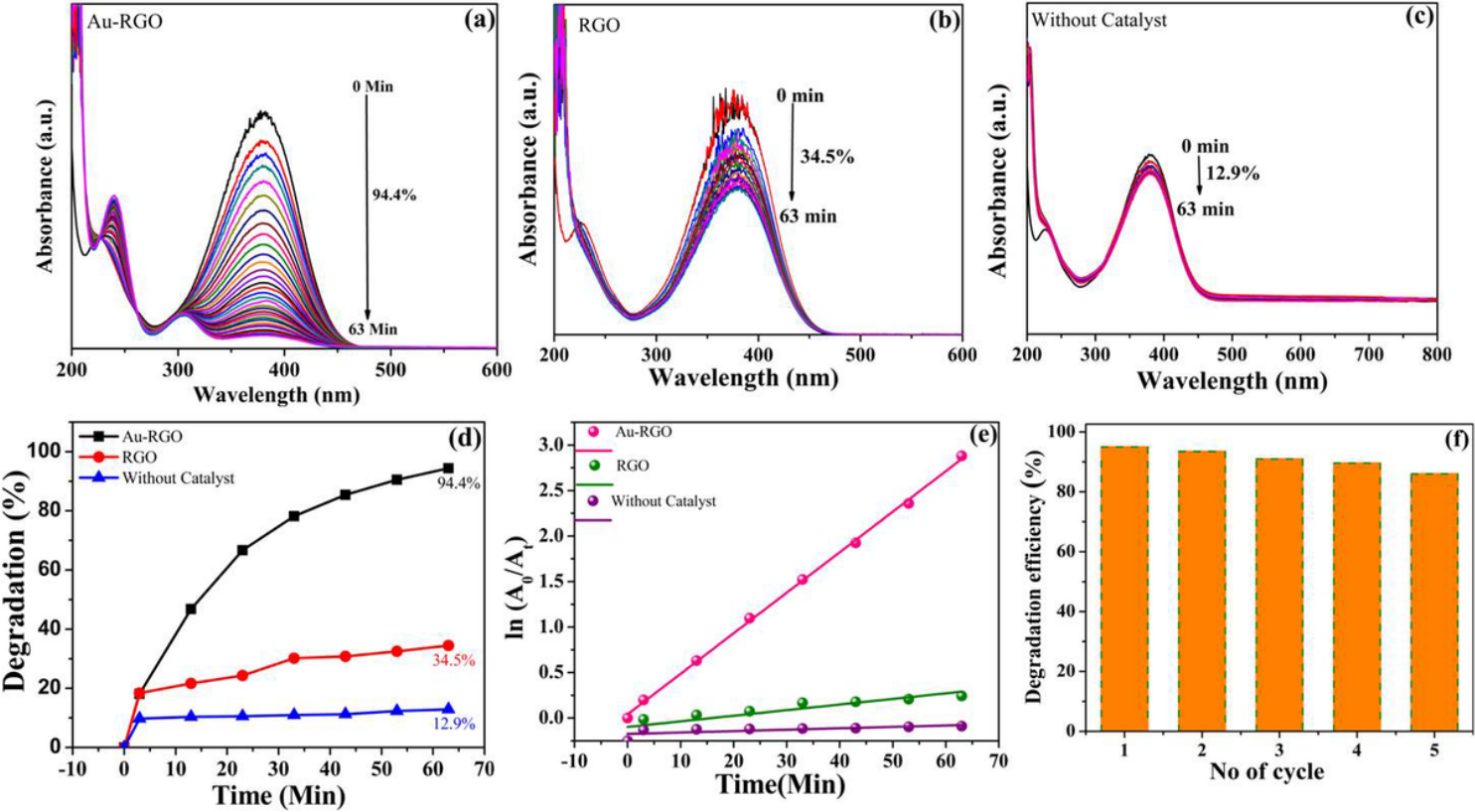


Figure 4

Absorption spectra of 4-NA solution in the presence of a Au-RGO, b RGO, c Without catalyst respectively. d Catalytic degradation of 4-NA and e rate kinetics of 4NA in the presence of Au-RGO, RGO and without catalyst f Recyclability tests of Au-RGO nanocatalyst for degradation of 4-NA dye

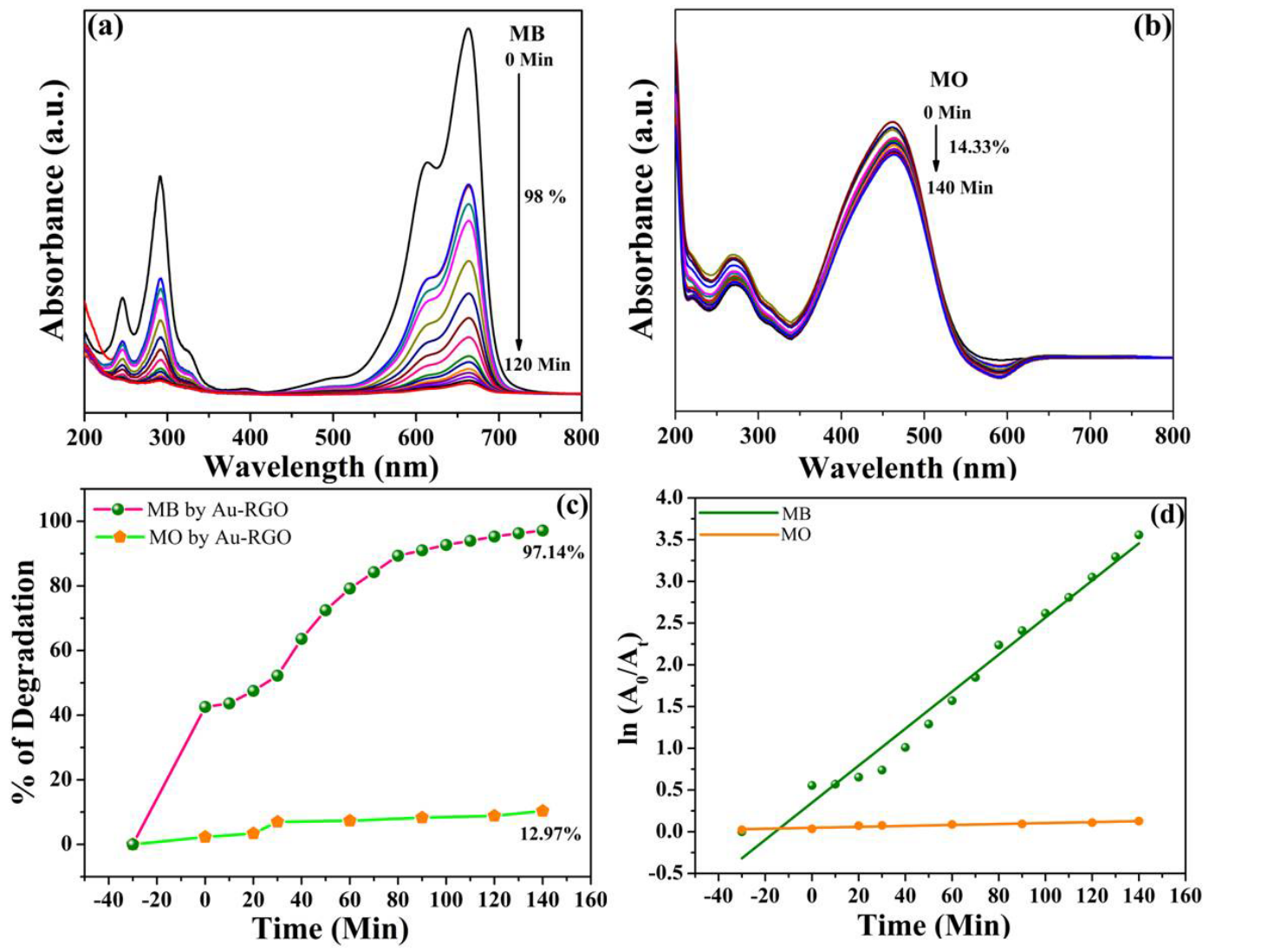


Figure 5
Absorption spectra of a MB solution b MO solution in the presence of Au-RGO under visible light irradiation. c Photocatalytic degradation of MB and MO d rate kinetics of MB and MO by Au-RGO photocatalysts under visible light irradiation

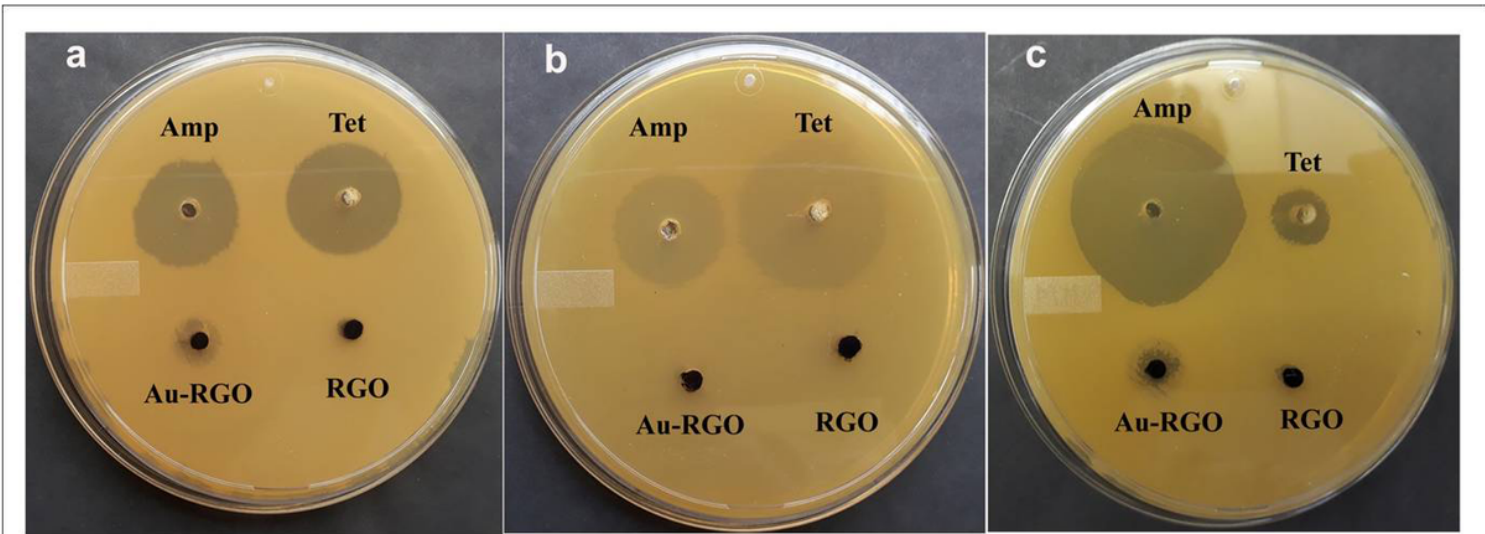


Figure 6

Antibacterial activity of Au-RGO and RGO against bacterial strains a *B. subtilis* b *E. coli*, and c *S. aureus*

Supplementary Files

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- [Scheme1.png](#)
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- [Scheme3.png](#)
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