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Biofabrication of polyphenols stabilized reduced graphene oxide and its anti-tuberculosis activity

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ABSTRACT

A facile one step eco-friendly method for the reduction graphene oxide by *Cinnamomum verum* (*C. verum*) bark extract is reported in this work. This approach avoids the utilization of hazardous chemical reagents. The characterization results of various spectroscopic and microscopic techniques for the prepared graphene oxide (GO) and reduced graphene oxide (RGO) afford a strong indication of the removal of oxygen functionalities of GO after reduction and following stabilization by the oxidised polyphenols. Fourier transform infrared spectral results showed the capping of oxidised polyphenols onto the surface of reduced graphene oxide which further prevent their aggregation. Additionally, the prepared graphene nanosheets were tested for their antituberculosis activity against standard strain such as *M. tuberculosis* H37Ra. The obtained results suggested that the synthesized graphene acts as an effective growth inhibitors against *M. tuberculosis* H37Ra making it applicable for targeted drug delivery by combining with other chemical drugs as a therapeutic index.

1. Introduction

Graphene, a new class of 2D nanomaterial have paid scientific due to its excellent chemical, electronic and physical properties [1]. Because of these extraordinary thermal, mechanical and electronic properties, reduced graphene oxide and its hybrid are considered as gifted materials in different fields of science and technology such as solar cells, sensors, polymer composites, field-effect transistors, and in biomedical applications [2–8].

From the last few years, various methods have been developed for the synthesis of RGO such as chemical reduction of GO [9–11], chemical vapor deposition [12] and micromechanical exfoliation of graphite. But, the synthetic approach of GO deoxygenation by chemical reagents is considered to be significant because of its industrial scale production with low cost. Various chemical reagents have been reported for the reduction of graphene oxide such as hydrazine [11], hydroquinone [13] and Sodium borohydride [14]. But, the utilization of these chemicals in large scale is harmful to the environment and human beings because of their hazardous and explosive nature. Hence, it is necessary to develop new environment friendly approaches for the efficient reduction of graphene oxide into RGO under mild reaction conditions.

Recently, several efforts have been made for the eco-friendly production of reduced graphene oxide using electrochemical, biological and microwave methods. Few green reductants such as Ascorbic acid, glucose, bovine serum albumin and amino acids have been reported for GO reduction, where they play role as deoxygenating and capping agents [15].

Recently, polyphenols such as gallic acid and tannic acid are proved to be acts reducing and stabilizing agents during the synthesis of RGO. Plant extracts contains different types of polyphenolic biomolecules containing phenolic hydroxyl groups which will be transformed to the corresponding quinone forms during the RGO synthesis. The subsequently formed oxidised polyphenols will stabilize the formed RGO by preventing the aggregation. The present work introduced an eco-friendly method of RGO preparation using an aqueous extracts of *C. verum* root as deoxygenating agents. Natural available polyphenols present in the *C. verum* extract are primarily accountable for the deoxygenation and stabilization of RGO nanosheets.

2. Experimental Section

2.1. Materials

Hydrogen peroxide (H_2O_2, 30%), Graphite powder (99.9995%, 100 mesh), Sodium nitrate ($NaNO_3$), H_2SO_4 (98%), $KMnO_4$, Rifampicin and all solvents were obtained from Sigma-Aldrich Chemicals Ltd.

2.2. Preparation of C. verum Extract

Initially *C. verum* root was separated and cleaned with distilled water and then allowed to dry under sunlight. About 1.5 g of finely

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dried powder of *C. verum* root was mixed with 100 mL of double distilled water and allowed to heat on a water bath at 80 °C for about 60 min. The resulting solution was cooled down to room temperature and filtered by using a cellulose nitrate membrane (0.2 μm) filter paper to obtain a clear solution of *C. verum* extract.

2.3. Preparation of Reduced Graphene Oxide

Graphene oxide, the precursor for RGO preparation was synthesized from graphite powder of 100 mesh size by following the reported procedure of modified Hummers method [16]. About 100 mL of *C. verum* extract was added to 100 mL of graphene oxide (1 mg/mL) in a 500 mL round bottom flask and thoroughly mixed well by shaking. The pH of the resulting mixture was maintained to 12 with the addition of NH_4OH. The subsequent reaction mixture was kept on a temperature controlled water bath and refluxed at 100 °C for about 12 h. The successful completion of GO reduction was indicated by color change of GO solution from brown to black. The formation of a precipitate of RGO at the bottom of the round bottom flask indicates the loss of oxygen functionalities of graphene oxide after 12 h treatment with extract.

2.4. Microplate Alamar Blue Assay (MABA) Assay

Initially, Rifampicin solution was prepared in DMF solvent as 10 mg/mL, and stored in a refrigerator at −20 °C. Anti-mycobacterial activity for the prepared graphene was carried out using the MABA assay. In brief, M. tuberculosis H37Ra colonies were collected from Lowenstein-Jensen (LJ) medium and added to 1 mL of double distilled water and the corresponding turbidity of the reaction solution was adjusted in order to match with McFarland tube No.1 (107 CFU/mL). The resulting solution was then diluted to 1:25 in 7H9 (Middlebrook 7H9 supplemented with 0.1% Casitone, 10% albumin dextrose and 0.2% glycerol, pH 6.8) and further used as inoculums. Later, about 100 μL of the bacterial suspension along with the graphene dispersion was then placed in each well in Middlebrook 7H9 medium and the total volume was adjusted to 200 μL and the total concentration of the graphene dispersions were 50 μg/mL, 100 μg/mL and 200 μg/mL. Simultaneously, a control experiment was also carried out with rifampicin as standard. A parafilm was used to seal the plates and finally incubated at 37 °C for about 7 days. After incubation, about 20 μL of Alamar blue dye was then added to all the wells and then incubated again for overnight at 37 °C. The change in the color of the solution from blue to pink further indicated the growth of bacteria. On the other hand, the lowest concentration of tested material that exhibited without color change was defined as Minimum inhibitory concentration (MIC). The MIC values of the graphene and Rifampicin were calculated. The acceptable MIC values of the drug were found to be in the range of 0.0047 to 0.0095 μg/mL.

3. Characterization

Fourier transform infrared (FTIR) analysis was done over the range of 4000–500 cm^{-1} by using a JASCO FTIR 4100 instrument. Samples

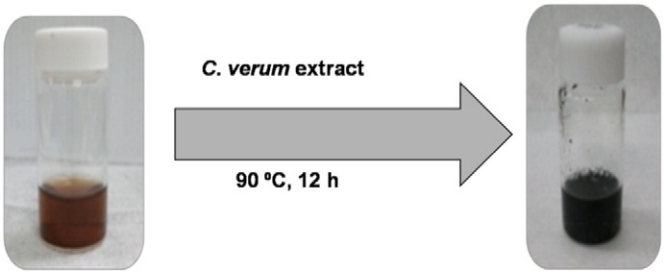

Fig. 1. Schematic illustration of *C. verum* extract induced reduction of graphene oxide.

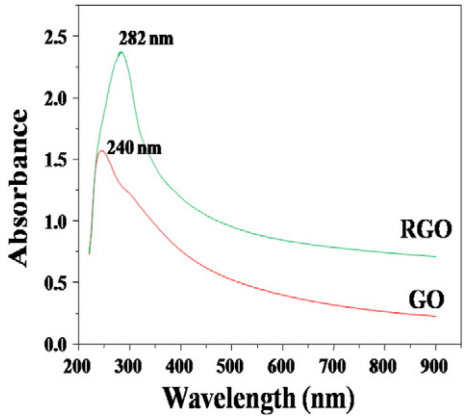

Fig. 2. UV–visible spectra of (a) GO (red) (b) RGO (green).

were pelletised by mixing little amount of RGO with KBr powder and analysed. UV–Visible absorbance measurements were carried out by using a Jasco V-670 UV–Vis spectrophotometer and the measurements were carried out by taking double distilled water as blank solution. A Bruker D8 Advance diffractometer was used for X-ray diffraction analysis. Instruments was run over 2θ of 3°–80° range with Cu Kα radiation of λ = 1.54A° with a scan speed of 4°/min and step size of 0.02°. Scanning electron microscopic (SEM) images for prepared RGO were taken by using a Carl Zeiss SEM instrument. The pulverised powder of RGO was placed over the carbon tape surface and images were taken under microscope. A Senterra R200-L apparatus (Bruker Optics) instrument was used for Raman analysis by using a laser wavelength of 532 nm. Additionally morphology of RGO was studied by using a JEOL-2100 F electron microscope instrument operating at 200 kV voltages. A much diluted dispersion of RGO was used for TEM analysis and simultaneous SAED measurements were performed. Malvern Instruments were used to take zeta potential measurements. A PHI Quantera SXM, scanning X-ray microprobe (ULVac-PHI Inc.) was used to take C1s XPS spectrum for the prepared RGO. A silicon wafer coated uniformly with RGO powder was used to take XPS measurements.

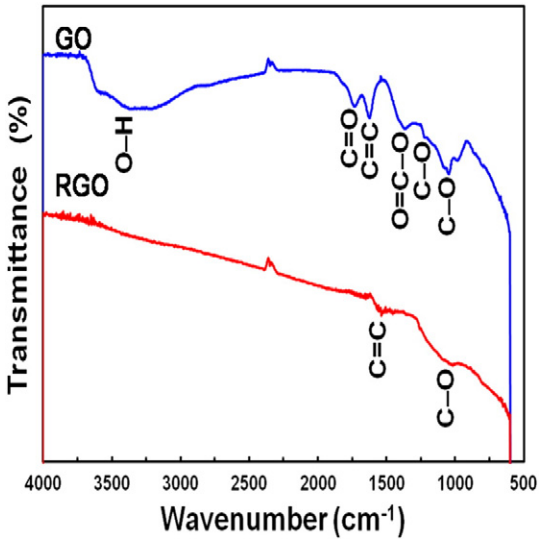

Fig. 3. FTIR spectra of GO (blue) and dried RGO (red).