



Sustainable Synthesis of Graphene-silver Nanocomposite Using Fenugreek Seed Extract for Potential Antioxidants

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Abstract: Graphene is a wonder material in the recent literature, mainly due to its outstanding physicochemical properties that enable its utility across a broad spectrum of applications, including electronics, sensors, catalysis, energy, drugs, biomedical implants, and diagnostics. Moreover, scalable production of graphene is achieved through green chemistry methods. Herein, a biogenic synthesis of reduced graphene oxide-silver (Ag-RGO) nanocomposite is reported by utilizing fenugreek seed extract as a sustainable reducing and capping agent. The structural changes during the reduction of graphene oxide with AgNO₃ to form an Ag-RGO nanocomposite are investigated using XRD, Raman, SEM, and TEM. SEM and TEM images displayed transparent graphene nanosheets adorned with silver nanoparticles (Ag NPs). Raman displayed both D and G bands that correspond to the RGO. XRD displayed representative peaks of both graphene and Ag NPs. Further, the RGO-Ag nanocomposite exhibited dose-dependent antioxidant activity against the DPPH radical, with activity increasing with increasing concentration. It is particularly noteworthy that the nanocomposite's antioxidant activity (81 % at a concentration of 500 µg/mL) surpasses the antioxidant efficacy of the standard compound, ascorbic acid (73 % at a concentration of 500 µg/mL). Therefore, the eco-friendly synthesized compound may find potential antioxidant applications in food packaging and medicinal drugs.

Keywords: graphene; biosynthesis; graphene-silver nanocomposite; antioxidants.

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1. Introduction

The novel properties of graphene have enabled its utility in a wide range of applications, and accordingly, the recent literature has witnessed a “graphene-driven gold rush” [1]. Reduced graphene oxide (RGO) and its composites have emerged as promising candidates due to their unique combination of mechanical, electrical, and chemical properties [2]. Incorporating

metallic nanoparticles into RGO matrices further enhances their functionality, opening avenues for diverse applications [3]. Silver nanoparticles (Ag NPs) exhibit remarkable catalytic, optical, antibacterial, sensing, Raman SERS, and other applications. Additionally, the antioxidant activity of silver has been explored for its potential in combating oxidative stress-related disorders [4]. However, the inherent instability and aggregation tendency of Ag NPs necessitate effective strategies for their immobilization and stabilization [5]. As a consequence, graphene-silver nanocomposites are extensively investigated for the above-mentioned applications. The large two-dimensional surface area of graphene ($\sim 2600 \text{ m}^2/\text{g}$) provides abundant sites for stable anchoring of various materials. Additionally, its extended π -conjugation and exceptionally high electron mobility ($\sim 200,000 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) facilitate effective electron transport and delocalization [6]. Hence, graphene synergises with the native activity of Ag NPs, resulting in an overall enhanced activity for the graphene-silver nanocomposite (which is more stable). These nanocomposites are generally synthesized using solution-based chemical methods, which are advantageous for bulk production at a lower cost than physical methods [7]. The chemical methods utilize hydrazine and its derivatives, sodium borohydride, etc., reagents in the reduction of graphene oxide (GO) and AgNO_3 to reduced graphene oxide (RGO), silver nanocomposites [8]. Thus, the obtained nanocomposites are further stabilized by employing additional capping agents in the subsequent steps [9]. But these hazardous chemical reducing agents are highly toxic, impart their toxicity to the synthesized nanocomposites, and require handling precautions [9]. In addition, their disposal triggers the environmental issues. Further, the poisonous nature of the obtained nanocomposites bars their use in biological and medicinal applications. Therefore, to alleviate these environmental and biological issues, researchers have embarked on biogenic synthesis techniques [10–12].

Green chemistry techniques utilize biomolecules, plant extracts, and natural products as effective reducing and stabilizing agents. These are envisaged as the best alternative to chemical methods because these reagents are naturally available, their disposal poses no pollution problems, do not require handling precautions, and the synthesized materials can be applied in biological and medicinal applications. The recent interest in green synthesis of graphene and its nanocomposites is attributed to their suitability for cost-effective bulk production, applicability in biological investigations, and *in situ* production. Amla, betel, custard apple, grapes, green tea, etc. [13–15]. Aqueous extracts are utilized in the biogenic synthesis of graphene-silver nanocomposites. Fenugreek seed extract (FSE) is used in the synthesis of graphene-palladium nanocomposite [16].

Fenugreek seeds belong to the Fabaceae family, which is formally called *Trigonella foenum-graecum*. Fenugreek seeds are brownish-yellow, cuboid, and extensively used in culinary applications owing to their rich mineral content and medicinal properties, particularly in China and India. Fenugreek seeds have been used traditionally for a variety of medical conditions, including diabetes, arthritis, and anti-cancer effects [17]. The phytochemical components, such as amino acids, alkaloids, flavonoids, saponins, and steroids, present in its aqueous extract have shown superior reducing and stabilizing properties in the biogenic synthesis of various metal, metal oxide, and graphene nanomaterials [17]. Due to the abundance of phytochemicals found in these seeds, including butanoic acid, eugenol, linalool, caproic acid, sotolone, etc. (Figure 1), the extracts from the seeds are used as stabilising and reducing agents when creating metallic nanoparticles. The use of FSE in the biogenic syntheses of gold nanoparticles (Au NPs) by Aromal *et al.* [18], graphene-Palladium nanocomposite by Chandan Singh *et al.*, [16], and Ag NPs by Awad *et al.* [17] has strongly proved that the

phytochemicals present in FSE are good reducing and stabilizing agents. Inspired by these findings, the FSE is used to create graphene-silver (Ag-RGO) nanocomposite, and its antioxidant properties are investigated in the present report. FSE is very rich in saponins, flavonoids, and other polyphenolic compounds, and their concentrations are generally higher than those of other leaf extracts. These biomolecules act as strong reducing and stabilizing agents, making the FSE more advantageous for green reduction technologies than other leaf extracts.

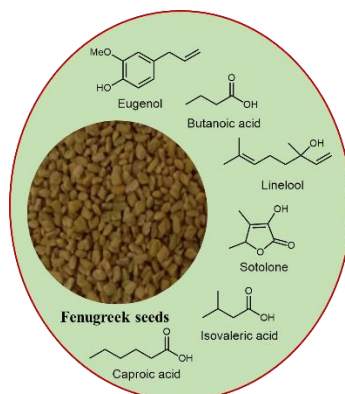


Figure 1. The phytochemical constituents of Fenugreek seed extract.

Protein structures, cell membranes, and DNA are all destroyed by reactive oxygen species (ROS), which also causes food to decompose and polymers to break down [19]. Antioxidants have numerous uses in the medical field, pharmaceutical business, cosmetics industry, and food sector because they scavenge ROS and prevent their harmful effects [19]. Due to their antioxidant properties, a number of carbon-based materials have been extensively studied, including graphene, fullerenes, carbon nanotubes, and carbon dots [20]. Effective antioxidant activity can be observed as a result of the sp^2 carbon centres in these carbon materials readily interacting with ROS to create an adduct or scavenge the radical, followed by rapid electron delocalization [20]. The RGO is a powerful radical scavenger due to its large 2D surface area and conjugated sp^2 carbon network. Furthermore, the combination of silver with graphene is expected to enhance the antioxidant properties [21]. Therefore, the green synthesis and the antioxidant activity of graphene-silver nanocomposite are investigated in the present report. In this context, the synthesis approach in this work is considered sustainable, as it avoids the use of toxic chemicals and relies on a renewable, plant-derived extract as both a reducing and stabilizing agent. The method operates under mild conditions without requiring harsh reagents or complex steps, thereby aligning with the principles of green chemistry. Such eco-friendly strategies significantly reduce environmental impact while enabling scalable nanomaterial production.

2. Materials and methods

2.1. Materials.

Chemicals such as graphite (Sigma-Aldrich), sulphuric acid, sodium nitrite, hydrogen peroxide, potassium permanganate, ethanol, dimethylsulfoxide, methanol, and DPPH of analytical grade were obtained from Avra Laboratories and used without further purification. We purchased fenugreek seeds from a nearby market. Double-distilled water was used to prepare all aqueous solutions.

2.2. Ag-RGO nanocomposite.

GO prepared by modified Hummers method was utilized [22]. Fenugreek seeds (10 g) were soaked in water for 1 h and then refluxed at $\sim 100^{\circ}\text{C}$ for 30 min. The reaction mixture was cooled to room temperature and filtered through Whatman filter paper to obtain the FSE. The precursor solution was prepared by dispersing 100 mg of GO in 100 mL of water via bath sonication; AgNO_3 (10 mg) was then added during the last 5 min of sonication. This precursor solution was combined with 100 mL of FSE and subjected to reflux for 12 h. During this time, the initial yellowish colour of the reaction mixture changed to black, indicating the formation of the Ag-RGO nanocomposite. The naturally cooled solution was filtered, washed multiple times with water, and then with ethanol. Finally, vacuum drying yielded Ag-RGO nanocomposite.

2.3. Characterization techniques.

Using a JASCO V-550 spectrophotometer, an ultraviolet-visible (UV-Visible) examination of the compounds was obtained within a 200–900 nm range. Using a Bruker D8 Advance diffractometer (USA), the X-ray diffraction (XRD) study was conducted within 2θ values of 10° to 100° . The source of the X-rays was $\text{Cu K}\alpha$ radiation ($\lambda = 1.54 \text{ \AA}$). The morphological images of the Ag-RGO nanocomposite were obtained on a TESCON VEGA3 scanning electron microscopy (SEM) instrument and a JEOL Japan JEM-2100 plus transmission electron microscopy (TEM) instrument. A Micro-Raman spectrometer (Raman-SR-TEC-IG, USA) was employed to access the Raman spectral data. The range in which the spectra were obtained was $200\text{--}3500 \text{ cm}^{-1}$.

2.4. Antioxidant activity.

Spectrophotometric measurements were made of the synthesised Ag-RGO nanocomposite (100–500 μg) to determine its antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Ascorbic acid was utilized as a standard for comparison. The absorbance of DPPH radicals was higher at 517 nm, and this absorbance decreased as an antioxidant was added. In summary, 100 μL of Ag-RGO nanocomposite solution at varying concentrations (100 – 500 $\mu\text{g}/\text{mL}$) in DMSO was combined with 900 μL of a 0.004% (0.1 mM) DPPH solution that had been produced in methanol. After completely vortexing the solutions and letting them sit at room temperature for 30 minutes, the absorbance at 517 nm was measured relative to a blank. The lower absorbances of these solutions gave a direct correlation with higher DPPH free radical scavenging activity. The antioxidant activity of the Ag-RGO nanocomposite was measured as the percentage of DPPH radical scavenging activity, as reported in the literature [23]. All the experiments were conducted in triplicate for reproducibility assessment, and the antioxidant values were presented as the mean of these measurements along with standard deviations.

3. Results and Discussions

3.1. XRD analysis.

The X-Ray Diffraction (XRD) studies of GO, RGO, and Ag-RGO depicted in Figure 2 were very vital in understanding the structural changes that occurred during this biogenic reduction. The GO exhibited a sharp peak at a 2θ value of 11° due to diffraction from the (002)

plane, which disappeared in the present reduction, and the corresponding RGO peak appeared at 25° [7]. The broadness was indicative of the presence of well-exfoliated graphene sheets. The same peak was observed at 25° in the Ag-RGO nanocomposite, confirming the formation of RGO in the nanocomposite. The other peaks, which are present at a 2θ value of 38° , 44° , 64° , and 77.3° , represent the (111), (200), (220), and (311) plane diffractions of face-centred cubic (FCC) silver [8]. Therefore, the effective biogenic conversion of GO and AgNO_3 into Ag-RGO nanocomposite by utilizing FSE was confirmed by XRD spectral data. The average particle size calculated using XRD data was 21 nm.

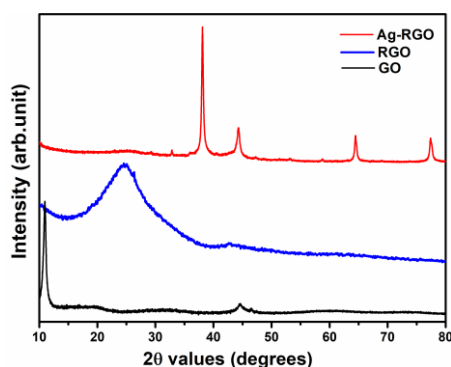


Figure 2. The XRD spectra of GO, RGO, and Ag-RGO nanocomposite.

3.2. Raman analysis.

The Raman spectral data of Ag-RGO nanocomposite were presented in Figure 3. The representative D and G bands of RGO appeared at 1322 and 1578 cm^{-1} , respectively, proving the presence of RGO in the Ag-RGO nanocomposite [24]. The D band was a representative of defective graphene, while the G band corresponds to pure graphene. The defects at edges were responsible for the D band, whereas zone centre E_{2g} phonon scattering would give the G band [24]. Thus, the Raman spectral data of Ag-RGO nanocomposite also confirmed the presence of RGO. Therefore, FSE successfully reduced the precursor solution into Ag-RGO nanocomposite. The intensity ratio of D and G bands (I_D/I_G) value was 1.2, and it was very close to the ideal value of 1 [24]. The I_D/I_G value of 1.2 was indicative of an amorphous nature, presence of vacancies, and the defects in the RGO [24]. This I_D/I_G ratio is a robust and quantitative measure of the antioxidant activity of rGO-based materials. A higher I_D/I_G ratio directly correlates with a higher density of structural defects and residual oxygen-containing functional groups. These defects act as the primary active sites for scavenging free radicals, thereby enhancing the rGO's antioxidant properties. Consequently, the I_D/I_G ratio serves as a reliable metric for assessing antioxidant activity, directly linking its structural disorder to its functional performance.

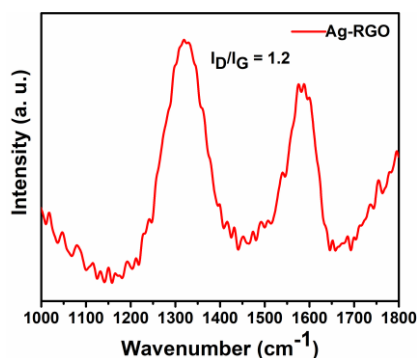


Figure 3. Raman spectra of Ag-RGO nanocomposite.

3.3. SEM and TEM analysis.

The morphological images of Ag-RGO nanocomposite obtained by SEM are presented in Figure 4a,b. These images clearly showed the transparent and wrinkled graphene sheets decorated with Ag NPs. Further, the EDX analysis (Figure 4c) showed peaks for carbon, oxygen, and silver, which once again confirmed the formation of the Ag-RGO nanocomposite and the strong reducing and stabilizing nature of the phytochemicals present in the FSE. The TEM images presented in Figure 4d-f further supported the SEM analysis. Graphene sheets of around 100-200 nm size were decorated with Ag NPs of sizes ranging from 60-80 nm. The graphene sheets were transparent with wrinkled and curved edges. Furthermore, the selected area electron diffraction (SAED) pattern presented in Figure 4g proved the hexagonal crystal structure of graphene carbons present in the present nanocomposite. Furthermore, the average particle size measured from TEM images is 73.15 ± 15.25 . The particle size distribution histogram from TEM images is presented in Figure 4h. Therefore, the Ag-RGO nanocomposite was successfully synthesized by reducing GO and AgNO₃ using FSE in a sustainable, biogenic method.

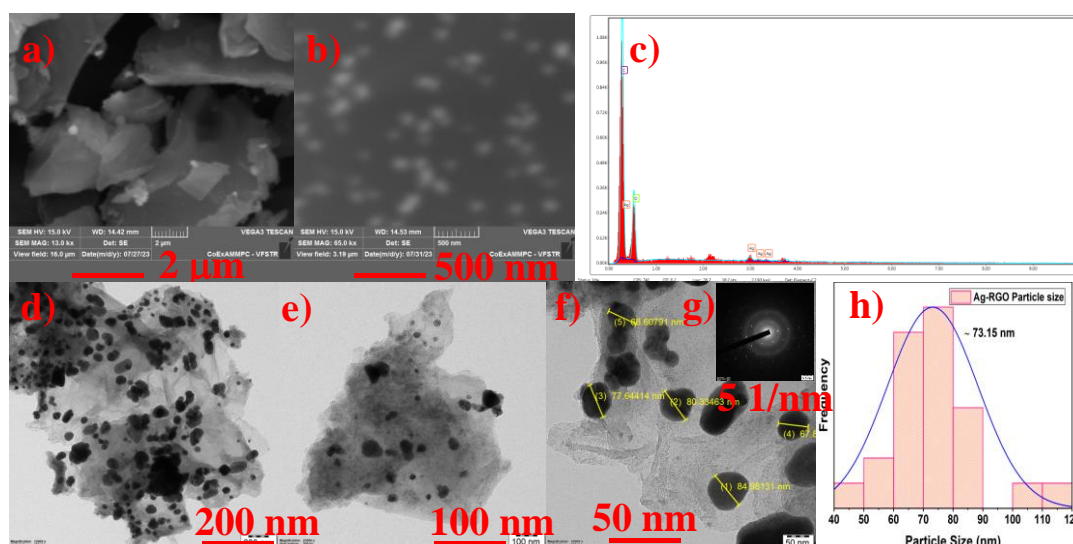


Figure 4. (a,b) Scanning electron microscopy images; (c) Energy dispersive X-ray (EDX) analysis of Ag-RGO nanocomposite; (d-f) Transmission electron microscopy images; (g) selected area electron diffraction (SAED) pattern; (h) Particle size distribution graph obtained from TEM images.

3.4. Antioxidant activity.

The antioxidant activity of the Ag-RGO nanocomposite (Figure 5) was evaluated by invoking the DPPH radical scavenging assay, with results compared to those of standard ascorbic acid (AA). Ag-RGO nanocomposite exhibited antioxidant activities of 42.49 ± 5 , 52.83 ± 2.25 , 53.71 ± 1.55 , 78.33 ± 4.96 , and 81.36 ± 5.49 % at varying concentrations from 100 to 500 μg/mL, respectively. At the same concentrations, AA exhibited 60.23 ± 1.19 , 64.58 ± 1.6 , 66.60 ± 7.27 , 71.29 ± 2.64 , and 73.46 ± 1.51 % of antioxidant activity only. Although at lower concentrations, the Ag-RGO nanocomposite exhibited lower antioxidant activity, at higher concentrations, its antioxidant activity was superior to that of the standard AA. Overall, the Ag-RGO nanocomposite showed a linear enhancement in its antioxidant activity with an increase in concentration and 11 % higher activity than AA at 500 μg/mL concentration. Therefore, the Ag-RGO nanocomposite exhibited a potential antioxidant activity at higher

concentrations, which may find applications in food packaging and medicinal fields. Furthermore, the IC₅₀ value of Ag-RGO nanocomposite was measured to be 186.32 µg/mL. The lower IC₅₀ value supports the potential antioxidant value of the Ag-RGO nanocomposite.

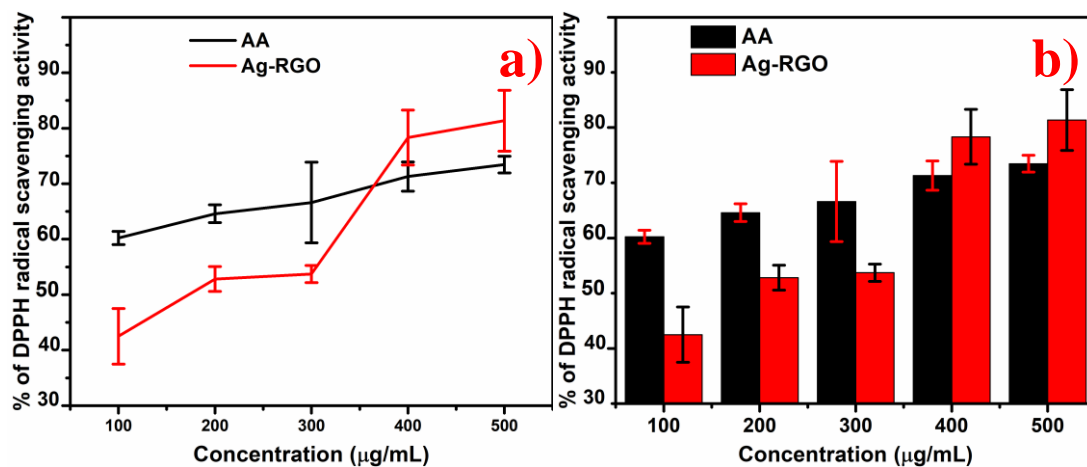


Figure 5. The comparative antioxidant analysis of Ag-RGO nanocomposite obtained by DPPH radical scavenging assay.

4. Conclusion

The recent interest in biogenic synthesis protocols for graphene was mainly driven by its scalable production and biological activity, which enable its utility across a wide range of applications. The synthesis of reduced graphene oxide-silver (Ag-RGO) nanocomposite was achieved by utilizing fenugreek seed extract as a sustainable reducing and capping agent. The formation of the Ag-RGO nanocomposite was confirmed by advanced spectroscopic techniques. XRD gave a peak of graphene at a 2θ theta value of 25°, and peaks that correspond to Ag NPs appeared at 38°, 44°, 64°, and 77°. Raman displayed both D and G bands at 1322 and 1578 cm⁻¹, respectively. The SEM images showed the wrinkled and transparent graphene sheets decorated with Ag NPs. Furthermore, the EDX analysis gave the peaks of C, O, and Ag in the Ag-RGO nanocomposite. Therefore, the phytochemicals of FSE were proven to be good reducing and capping agents. Thus, the synthesized Ag-RGO nanocomposite demonstrated a dose-dependent DPPH radical scavenging activity, surpassing that of ascorbic acid at higher concentrations. The nanocomposite showed superior antioxidant activity of 81 % (11 % higher than the AA) at 500 µg/mL concentration. Hence, the present biogenic synthesis protocol may be extended to the synthesis of other metal NPs and graphene composites. The synthesized Ag-RGO nanocomposite may find potential applications in food packaging and medicinal fields due to its superior antioxidant activity.

In this context, the synthesis approach in this work is considered sustainable, as it avoids the use of toxic chemicals and relies on a renewable, plant-derived extract as both a reducing and stabilizing agent. The method operates under mild conditions without requiring harsh reagents or complex steps, thereby aligning with the principles of green chemistry. Such eco-friendly strategies significantly reduce environmental impact while enabling scalable nanomaterial production.

Author Contributions

Conceptualization, R.V.N., D.R. and N.P.; methodology, A.R., R.V.N., D.R. and N.P.; validation, A.R., K.V.L. and A.R.R.; formal analysis, A.R., K.V.L., D.U.S.C., B.M.K.K and <https://nanobioletters.com/>

R.V.N.; investigation, A.R., A.R.R., D.U.S.C. and R.K.; data curation, A.R., K.V.L., B.M.K.K and B.C.; writing—original draft preparation, A.R., R.V.N., A.R.R. R.K. and B.C.; writing—review and editing, K.V.L., D.U.S.C., R.K., B.C.; supervision, R.V.N., D.R. and B.C.; project administration, N.P., R.K., B. M. K. K. and B. C.; funding acquisition, B.C. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflict of Interest

The authors declare no conflict of interest.

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