SHORT COMMUNICATION

Green Synthesis of Reduced Graphene Oxide using *Diplazium maximum* (D. Don) C. Chr.

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Abstract

Graphene is important due to its mechanical, electrical, thermal, nontoxic, and extraordinary chemical stability, and high surface area properties. Derivative of graphene, i.e., rGO, can be synthesized by using chemical methods as well as green methods. The present study focuses on synthesizing reduced graphene oxide (rGO) using young fronds of *Diplazium maximum*. Sustainably synthesized rGO was characterized using UV-visible spectroscopy, X-ray diffraction (XRD), and FTIR analyses. UV-visible spectroscopy revealed increased conjugation as the Graphene oxide was treated with bioactive plant extract, indicating the conversion of GO into rGO. Infrared (IR) analysis indicated the disappearance of several peaks and a fall in the intensity of certain peaks, supporting the successful synthesis of rGO. Additionally gradual disappearance of certain diffraction peaks and the appearance of other peaks in X-ray diffraction (XRD) analysis confirmed the successful synthesis of rGO by using *D. maximum* phytoextract.

Results successfully demonstrated that the synthesis of reduced graphene oxide using the young frond of *D. maximum* was lucrative, renewable, and environmentally friendly.

Keywords: Graphene oxide, Reduced graphene oxide, Green synthesis, *Diplazium maximum*.

Highlights

Significantly reduction of graphene oxide (rGO) using young fronds of *Diplazium maximum* plant extract.

Synthesized rGO was characterized using UV-visible spectroscopy, X-ray diffraction (XRD), and FTIR analyses.

The shift in peak position from 235nm in GO to 275nm in rGO, the absence of characteristic GO peaks in the rGO, indicated the synthesis of reduced graphene oxide.

In X-ray diffraction, the decrease in d-spacing indicates that the treatment has reduced water molecules and other oxygen functionalities.

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Introduction

A carbon allotrope is a graphene single-layered nanostructure, constructed by the combination of sp^2 hybrid carbon atoms in a hexagonal honeycomb-like lattice with a molecular bond length of 0.142 nm (Tai *et al.,* 2018).

Due to its various versatile properties such as high surface area, good electrical and thermal conductivity, and high mechanical strength graphene and its derivatives are used in different developing areas, especially in nanotechnology, energy storage, and bio-sensing (Lu *et al.,* 2009), drug delivery (Sun *et al.,* 2008), supercapacitors, and catalysis (Song *et al.,* 2010; Tamang *et al.,* 2023).

Deoxygenation of graphene oxide (GO) produces reduced graphene oxide (rGO) through different chemical and physical processes (Wang *et al.,* 2011; Tamang *et al.*, 2023). Chemical-reduction methods are initially hailed for their large-scale production, but have potential risks. Various reducing agents such as hydrazine, hydroquinone, sodium borohydride, and hydrogen sulfide are corrosive, hazardous, and harmful. Due to these disadvantages, an eco-friendly, plant-based, sustainable green reduction method was proposed to produce rGO (Zhang *et al.,* 2010). Plants contain various compounds that can act as reducing agents, facilitating the reduction of graphene oxide (GO) to graphene or reduced graphene oxide (rGO) (Rikhari *et al.,* 2019).

A large number of plant sources such as green tea (Wang *et al.*, 2011), mango leaves (Sadhukhan *et al.*, 2016), Aloe vera (Bhattacharya *et al.*, 2017), Eucalyptus bark (Manchala *et al.*, 2019), kaffir lime peel (Wijaya *et al.*, 2020), rose water (Perumal *et al.*, 2022), sugarcane bagasse (Gan *et al.*, 2019), *Tinospora cordifolia* (Saini *et al.*, 2024), lemon Juice extract (Mahiuddin & Ochiai, 2021), *Bougainvillea glabra* (Das *et al.*, 2023), *Vernonia amygdalina* (Meka Chufa *et al.,* 2021), *Tecoma stans* (Mahmoud *et al.*, 2022) etc. are frequently used bio reductant to reduced graphene oxide.

Several reports in the literature describe the various applications of green-synthesized rGO using different plant species, such as rGO synthesized from *Phaseolus aureus* L. had a high specific capacitance and excellent electrochemical cyclic stability (Jana *et al.*, 2015). Similarly, Punniyakotti *et al.* (2021)

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Fig. 1: Young frond of *D. maximum*

investigated the reduction of GO using *Acalypha indica* and *Raphanus sativus* and investigated the anticancer activity against human breast (MCF-7) and A549 cancer cell lines. Yaragalla *et al.* (2016) demonstrated the antimicrobial and anti-proliferation activity of rGO synthesized using grapes. Wang *et al.* (2020) established the synthesis of rGO from *Memecylon edule* and demonstrated it as a photothermal therapeutic agent for cancer cell apoptosis.

Diplazium maximum (D. Don) C. Chr. is a popular edible wild fern that is a rich source of various phytoconstituents, including polyphenols, flavonoids, polyunsaturated fatty and dietary fiber (Sareen *et al.,* 2021). Young fronds of *D. maximum* have been significantly consumed as vegetables (Thakur *et al.,* 2017; Rana *et al.,* 2019). Recently, Sareen *et al*. (2021) demonstrated that this species has effective antioxidant potential. To the best of our knowledge, no research is available on the utilization of *D. maximum* plant extract for reducing GO.

Based on our current understanding and the results of this investigation, we synthesized the reduced graphene oxide using *D. maximum* extract for the first time. This research would be a strong foundation for further investigation into the application of the synthesized product.

Materials and Methods

Chemicals

Graphite powder purchased from SDFCL, potassium permanganate purchased from CDH Pvt. Ltd, sodium nitrate purchased from NICE Chemical Pvt. Ltd, sulphuric acid purchased from AVARICE laboratory Pvt. Ltd, HCl purchased from RANKHEM.

Collection of plant material

Young frond of *D. maximum* was obtained from the Central Himalaya in India (1627 masl; 29.58 N, 80.22 E) following Jain and Rao (1977) (Fig. 1). Taxonomic identification was confirmed by the Department of Botany, L.S.M. Campus Pithoragarh, and the herbarium of the plant was also deposited in the department, for further reference.

Preparation of extract

The bulk mass of the fresh young fronds of *D. maximum* was brought into the laboratory, thoroughly washed with tap water to remove the adherent dust particles, and shade-dried at room temperature. Dried leaves were ground into fine powder using an electric grinder. Powdered material (24 g) was soaked in 100 mL de-ionized water and refluxed for about 40 minutes at 80–100℃ temperature. After this, the mixture was filtered using Whatman's filter paper no.1 (pore size 110 mm). The filtrate (phytoextract) was stored at 4℃ for further experimental analysis (Perumal *et al*., 2022).

Synthesis of graphene oxide (GO)

Modified Hummer's method is selected to synthesize the graphene oxide (GO) from graphite powder (Zaaba *et al*., 2017). With constant stirring, 50 mL of concentrated sulphuric acid (H_2SO_4) was mixed with 2.0 g of graphite powder and 1.0 g of sodium nitrate ($NaNO_3$). After 1 hour, 6 g of potassium permanganate ($KMnO_4$) was added slowly to the above solution by keeping the temperature below 20℃ to minimize or stop the overheating or explosion. This mixture was stirred by a magnetic

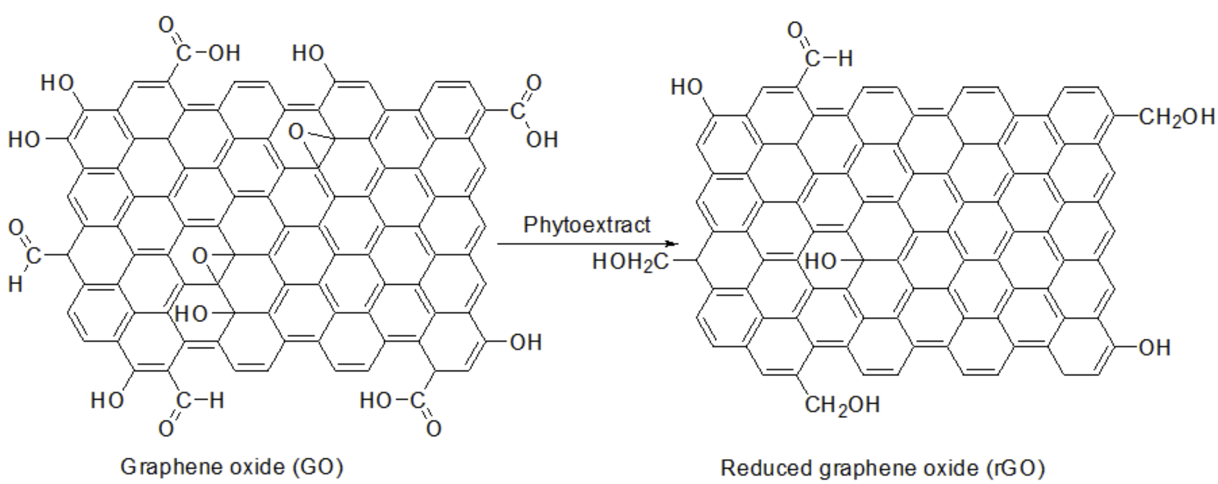

Fig. 2: Reaction showing the synthesis of rGO by *D. maximum*

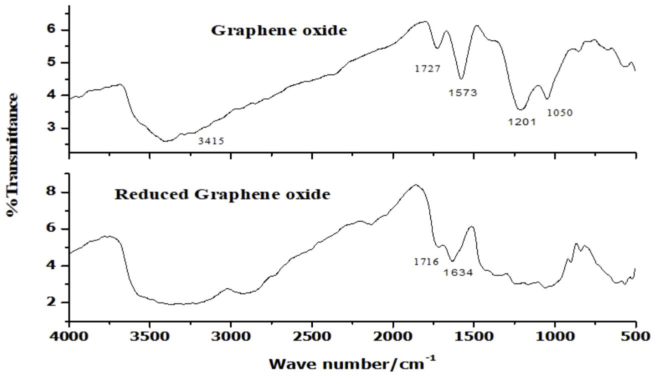

Fig. 3: FTIR spectrum of GO and green-synthesized rGO

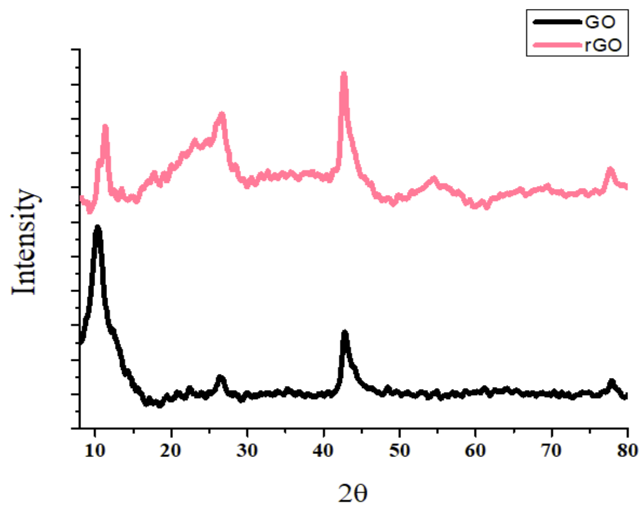

Fig. 4: X-ray diffraction (XRD) pattern of GO and green synthesized rGO

stirrer at 35℃ for 12 hours, after that the solution was diluted with 1L of de-ionized water with fast stirring. The suspension was treated with a 30% H_2O_2 solution (10 mL). In the end, the solution was finally washed with HCl and de-ionized water, respectively (three times each), followed by filtration and drying, giving graphene oxide (GO) (Shahriary and Athawale, 2014).

Reduction of Graphene oxide (GO) by phytoextract

Synthesis of reduced graphene oxide (rGO) from graphene oxide (GO) can be done by using a chemical reduction method and a green approach (with the help of phytoextract). For the synthesis of reduced graphene oxide (rGO) from graphene oxide (GO) with the help of phytoextract, 0.06 g of GO powder was mixed with 25 mL de-ionized water and ultrasonicated for 1 hour at room temperature to make a stable GO suspension. Then 25 mL of phytoextract was mixed with GO suspension (keeping a 1:1 volume ratio of graphene oxide and phytoextract) and magnetically stirred for 12 hours at 60℃. The reaction mixture was heated at 70 to 80℃ under refluxed conditions in an oil bath for 8 hours, reduction of graphene oxide was confirmed through the detection of a color change from black to brownish yellow. The mixture was allowed to cool at room temperature, then centrifuged, and finally, the product was collected after repeated washing with hot water followed by alcohol. The rGO obtained was dried in vacuum desiccators at room temperature for 48 hours (Perumal *et al*., 2022).

Physical characterization

Characterization of rGO was carried out through UV-visible spectra, XRD patterns and FTIR analysis.

Results and Discussion

The derivative of graphene, i.e., reduced graphene oxide (rGO), synthesized through the mixing of graphene oxide (GO) with phytoextract of the young fronds of *D. maximum,* is illustrated by the following reaction:

The observed change in color from black to brownish-yellow is indicative of the conversion of GO into rGO, driven by an augmented degree of conjugation. This transformation is corroborated by the analysis of UV-visible spectra, where a discernible shift is noted in the peak corresponding to π-π* transitions, shifting from 235 nm in GO to 275 nm in rGO. This shift in peak position signifies an augmentation in planarity due to the heightened conjugation within the rGO structure. Furthermore, the peak originally associated with n→π* transitions at 343 nm in GO assumes a shoulder-like feature in the UV-visible spectrum of rGO, indicative of a reduction in carbonyl functionality during the conversion processes (Fig. 2) (Mahiuddin and Ochiai, 2021; Yang *et al*., 2021).

The IR spectra of oxidized GO and rGO reveal distinct differences. In the GO spectrum, several key features are observed, including a broad band at 3415 cm^{-1} corresponding to O-H bond stretching, a sharp peak at 1727 cm^{-1} indicative of C=O stretching, and a peak at 1581 cm^{-1} arising from oxidized C=C stretching in carbonyl groups. Additionally, there are distinct peaks at 1201 and 1050 cm^{-1}, which represent C-O stretching for epoxide and alcohol functional groups, respectively. Conversely, the IR spectrum of rGO exhibits noteworthy changes. A novel peak emerges at 1628 cm^{-1}, attributed to the stretching of planar C=C bonds, signifying aromatization. Furthermore, the intensities of all the aforementioned peaks in rGO are notably reduced, suggesting a reduction in the presence of functional groups. Most importantly, the absence of characteristic GO peaks in the rGO IR spectrum signifies that the reduction of GO was facilitated by the bioactive constituents found in the extract of the young frond of *D. maximum*, highlighting the green and eco-friendly nature of this reduction process (Fig.3) (Mahiuddinand Ochiai, 2021; Yang *et al*., 2021).

X-ray diffraction (XRD) is a crucial technique for identifying phases and determining crystalline structure parameters. In the case of GO, a 2θ value of 10.22 indicates the successful oxidation of graphite into graphene oxide. This process introduces various functional groups onto the graphene, such as epoxide, carboxylic acids, and alcohols. Upon treating GO with the *D. maximum* extract, a new broad peak emerges at 2θ = 26.72. This peak is attributed to the restacking of graphene layers, resulting in reduced interlayer spacing or d spacing. The decrease in d-spacing indicates that the treatment has reduced water molecules and other oxygen functionalities from the graphene plane. Additionally, both spectra exhibit a small peak at $2\theta = 42.76$, which suggests the presence of graphene planes in the material (Fig. 4) (Mahiuddin and Ochiai, 2021).

Conclusion

It is noteworthy that the current study is the first report on the synthesis of rGO using *D. maximum* extract. UV-vis spectroscopy, IR spectroscopy, and XRD analysis collectively demonstrate the successful synthesis of rGO using a sustainable green approach in place of the chemical reduction method, which is hazardous and involves the wastage of chemicals that leading to a non-environmentally friendly approach. The utilization of this plant extract as a reducing agent has proven to be efficient, cost-effective and environment friendly green approach. Further investigations still need to be conducted on the toxicity of the product and biocompatibility analysis to effectively exploit human health benefits.

Conflict of Interest

The authors declare that they have no conflict of interest.

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