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GREEN SYNTHESIS OF REDUCED GRAPHENE OXIDE SILVER NANOCOMPOSITE USING *Anisomeles malabarica* (L.) R. BR. LEAF EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

The development of new antimicrobial materials has arisen in response to the need to prevent and control the growth of pathogenic microorganisms without the use of antibiotics. The Silver (Ag) nanoparticles attached r-GO (rGO/Ag) nanocomposite was synthesised in the present work using a one-pot *in-situ* chemical reduction approach and studied for antibacterial property**.** We developed a rapid preparation of graphene from graphite using modified Hummers method and reduced by *A. malabarica* leaf extract. UV-Vis spectroscopy and FT-IR were used to characterize the synthesized GO and rGO–Ag nanocomposite. The antibacterial properties of the synthesized GO and rGo-Ag nanocomposite were evaluated against human pathogenic organisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* using agar well diffusion method. At 50µl concentrations, synthesised GO inhibited *Pseudomonas aeruginosa* (19 mm) and rGO-Ag nanocomposite inhibited *Staphylococcusaureus* (28 mm).Antibacterial properties of the nanocomposites were found to be efficient against both gram positive and gram negative bacteria. Thus, green synthesized RGO-supported Silver nanoparticles play an important role in the medical disciplines.

Keywords: Antimicrobial, Graphene Oxide, rGO –Ag nanocomposite, Gram Negative Bacteria, Gram Positive Bacteria.

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INTRODUCTION

Nanotechnologists have been interested in graphene-based materials because of its outstanding crystal and electrical properties. The new nanomaterials are variety of exciting applications and also used widely in various fields including electronics, energy storage, biosensorsand biomedicine.[1-2] The industrial applications of graphene depend upon its availability of economical efficient and feasible method for allowing the preparation of graphene on large scale.[3] The reduction of graphene from graphene oxide is a thermal, electrochemical or chemical process is produced the reduction of graphene oxide. Chemical reduction is a one of the good approaches for a mass production of graphene because it's an economical and it's a low demand for equipment and simple process.[4] These reducing chemicals are highly toxic, hazardous and it allows introducing impurities to the reduced graphene oxide may be a harmful to the environment and has an adverse effect on its biological applications.[5]

Synthesis of graphene from graphene oxide is a one of the growing fields in biomedical industry. The use of microorganisms and plants to biologically reduce graphene oxide has been proposed as a possible environmentally benign alternative to producing graphene oxide. Plant based methods for reduction of graphene oxide is one of the greatest advantages over microorganisms based method.[6]Despite these benefits, no research on plant screening and reaction conditions optimization has been documented, indicating that plant extracts can effectively reduce graphene oxide. Although the unique and first way of appealing off graphene layers from graphite is exciting and useful for fundamental science, it is less efficient for high yield graphene synthesis. Synthesis of graphene particles are also reported to possesses non toxic effects and hence it can be widely used in the field of medicinal research.

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Anisomeles malabarica(L.) R. Br. (Labiatae) is found throughout tropical regions of India. It is an erect shrub, commonly known as Malabar catmint and commonly found at foot hills of Western Ghats. This plant is used in folk medicine from traditional times. The essential oil present in the herb is used for uterine affection. Traditional uses of *A. malabarica* includes treating tremors, convulsions, psychosis, stiffness of thigh, muscle cramps, headache, eye disorders, arthiritis, improves the quality of blood, diseases of gums and teeth, earache, vomiting, cough, cold, fever, asthma and wounds.[7] *A. malabarica* is reported to have antibacterial, antipyretic, analgesic, anti-inflammatory, antiallergic, anthelmintic, antiseptic activities and it also act as natural herbicide in wheat fields.[8] The plant *A. malabarica* is used to change the physical, chemical or biological properties of water and used in the purification of water.[9] This study aims to explore the mechanism of antibacterial activity of GO and rGo-Ag nanocomposite.

EXPERIMENTAL

Selection and Collection of Plant Material

A. malabarica was collected from sakthi mills in Pollachi taluk, Coimbatore, Tamilnadu. The plant was identified by PG and Research department of Botany, NGM College, Pollachi.

Preparation of Leaf Extracts

Shade dried powder of *A. malabarica* 10 g was mixed with 100 ml of distilled water. It was boiled for 10 minutes and the extract was stored at 4^0C for a week before being utilized.

Preparation of Graphene Oxide

A modified Hummer's method[10] was used to produce graphene oxide from graphite powder. 2g of graphite powder was added to 46ml of conc. Sulphuric acid in ice path and 6g of Potassium permagnate was progressively added to avoid heating and the resulting liquid was agitated for 2 hours (if an temperature increased for 35^0C an addition of 2 hours in stirrer condition). After an hour, the mixture was diluted in 280 ml of deionized water and 10 ml of hydrogen peroxide (30%). The change in color from dark brown to yellow was observed. This suspension was allowed to settle for one day. The clear supernatant was decanted and the residual suspension was filtered and washed with 5% of hydrochloric acid solution and acetone, followed by multiple washes with distilled water to remove the acid. A finally synthesized graphite oxide paste was freeze dried at -50^0C for one day. Synthesis of graphite oxide to graphene oxide was achieved by Ultra-sonication for an hour, the dispersion was centrifuged for 3000 rpm for 10 minutes, finally the resulting supernatant was centrifuged for 15000 rpm for 15 minutes and it was freeze dried for one day. Finally the aqueous dispersion containing graphene oxide was obtained.

Preparation of Graphene Oxide in Ag Suspension

Adding to the modified method of Cui *et al.*[11] (2014) 0.1M Silver Nitrate was added to 1ml of Graphene oxide suspension and kept in stirrer for 2 hours then the suspension was centrifuged to remove the Ag^+ ions. The precipitate was rinsed with distilled water and re-suspended in15ml distilled water.

Reduction of Graphene Oxide Ag Suspension into Ag-RGO nanocomposite Using the Leaf Extract

About 4 ml of plant extract added to the suspension of Go-Ag and kept in stirrer for 4 hours at 70^0C.

Characterization of Graphene Oxide Nanocomposite

The experiments were carried out in deionized water with aqueous graphene oxide and reduced graphene oxide. The absorbance between 300 and 700 nm was measured in 1ml of blank and 1ml of reaction mixture for UV-VIS spectra analysis. FTIR spectrum with a resolution of GRO in the range of 4000-400 cm-1 displays various bands that suggest the presence of phytomolecules. The samples were combined with KBr, and a thin sample disc was formed by pressing using a disc forming machine before being placed in the Fourier Infrared (FTIR) for graphene particle characterization.

Antibacterial Activity (Well Diffusion Method)

The antibacterial properties of the synthesized GO and rGo-Ag nanocomposite were evaluated against human pathogenic organism like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* using agar well diffusion method.

RESULTS AND DISCUSSION

Graphene oxide was initially made by using the modified Hummers process to oxidise pure natural flake graphite (NFG). The graphite oxide was then exfoliated in distilled water in an ultrasonicator to produce graphene oxide (GO). Later, GO was thermally reduced to obtain reduced graphene oxide (rGO). For the synthesis of GO, a modified Hummers method was applied. The oxidation of the NFG produces a thick brown sludge. The slurry contains graphite oxide and exfoliated sheets, as well as unoxidized graphitic particles and residual oxidizing agents. Salts and ions from the oxidation process were removed from the slurry using dialysis. Figure-2 depicts a graphene oxide (GO) sample made from graphite. GO is brown in colour and has a yellowish color in low concentrations.

UV-Visible Spectral Analysis

Figure-1 depicts the ultraviolet-vis spectra of aqueous GO dispersions. The peak value showed at 309 nm which confirm the Graphine oxide. Lai *et al*.[12] reported the same result in UV- vis spectral analysis of GO. The absorption spectra of the rGO–Ag nanocomposite showed a new peak at 323 nm after the deposition of AgNPs on the rGO surface; the band at 323 nm was attributed to surface plasmons and shows the existence of AgNPs in the rGO nanocomposite (Fig.-2). The elimination of the GO characteristic peaks and the creation of a new band arising from AgNPs plainly suggest that both GO and AgNO3 were simultaneously reduced, resulting in the production of the rGO–Ag composite. Under alkaline circumstances, Pasricha et al.[13]synthesised Ag–GO nanocomposites. Sangiliyandi *et al*.[14] reported the simultaneous reduction of both GO and Ag ions using *Tiliaamurensis* plant extracts as the reducing agent. In the present study *Anisomeles malabarica* plant extract also showed similar result.

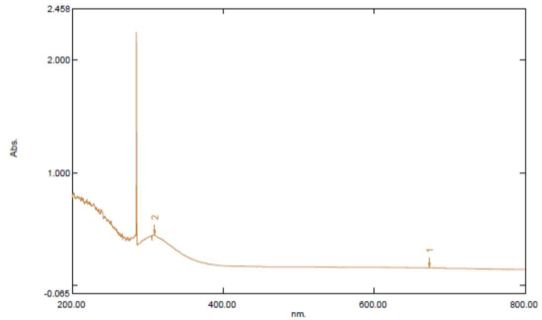

Fig.-1:Ultravoilet–visible Absorption Spectra of GrapheneOxide

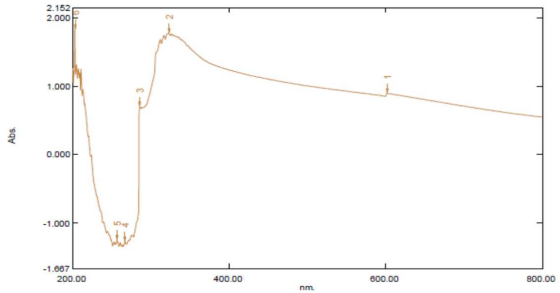

Fig-2: Ultraviolet–visible Absorption Spectra of rGONanocomposite

FTIR Spectroscopy

The structure and functional groups of GO and rGO nanocomposite were investigated using FTIR analysis. Figure-3 shows the FT-IR spectrum of GO, which indicates the presence of oxygen-containing

groups like functional hydroxyl, epoxy, and carboxylic groups. The aromatic C=C group's peak at 1571.99 cm^{-1} was visible in both GO and rGO-Ag nanocomposite samples (Fig.-4). The stretching vibration of C=C is responsible for the presence of an absorption peak at 1639 cm^{-1} and 1637 cm^{-1} in the medium frequency region of the spectra of both GO and rGO nanocomposite. The band near 1055 cm^{-1} is commonly linked to C–O (epoxy) groups, whilst the band at 1205 cm1 is usually attributed to C–OH stretching vibrations. Marcano *et al*.[15] discovered similar function groups of O-H, C=C, and C–O stretching vibrations in produced graphene oxide. The presence of O-H, C=O, C–O stretching vibration also found in synthesized Graphene oxide. The O–H stretching vibrations of the C–OH groups and water are responsible for the significant peak about 3500–3800 cm^{-1}.[16]

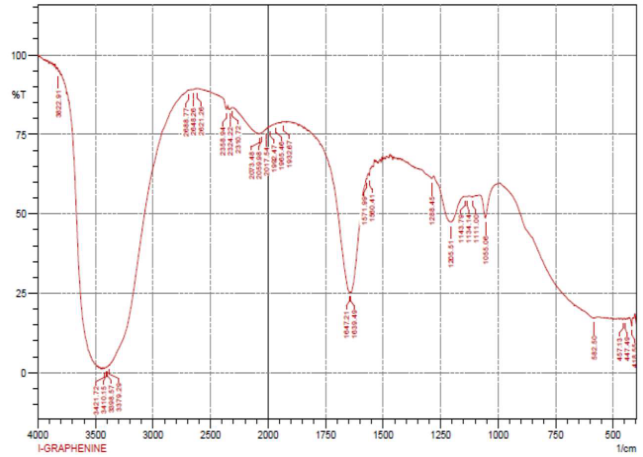

Fig-3: FT-IR Spectra of Synthesized Graphene Oxide

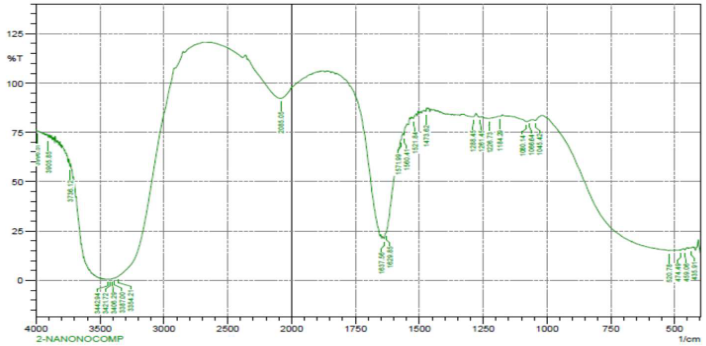

Fig-4: FT-IR Spectra of Synthesized rGo-Ag Nanocomposite using *Anisomeles malabarica* extract

Antibacterial Analysis of GO and rGO-Ag Nanocomposite

The antibacterial activity of the GO and rGO-Ag nanocomposite against Gram negative and Gram positive microorganisms was showed in Table-1. At 50 µl concentration, the synthesised GO inhibited *Pseudomonas aeruginosa* with a maximum zone of inhibition of 19 mm, followed by *E. coli* (18 mm), *K. pneumoniae* (18 mm) and *Staphylococcus aureus* (14 mm). The synthesized rGO-Ag nanocomposite showed the maximum zone of inhibition 28 mm against *Staphylococcus aureus, ,* which were followed by *Pseudomonas aeruginosa* (20mm), *E. coli* (17mm) and *K. pneumoniae* (17 mm) at 50 µl

concentration (Fig.-5). The GO and rGo nanocomposite samples showed highest zone of inhibition against all the tested organisms. This is much higher than that of standard ampicillin at 25 µl (10 mg/ml) except *E. coli*. There was a significant difference in zone diameter between GO and r GO-Ag nano composite.

Table -1: Antibacterial Analysis of GO and rGO-Ag Nanocomposite

S. No.	Organism	Zone of Inhibition(mm)			
		rGO-Ag (50 µl)	GO (50 µl)	Ampicillin	Distilled water (50 µl)
1	*E. coli*	17	18	17	0
2	*K. pneumonia*	17	18	19	0
3	*S. aureus*	28	14	20	0
4	*P. aeruginosa*	20	19	18	0

The rGO-Ag nanocomposite obtained from *Cardiospermum halicacabum* was studied for antibacterial activity against four pathogenic strains *P. aeruginosa, K. pneumoniae, S. aureus* &*E.coli*. *P. aeruginosa* has the largest zone of inhibition, while *K. pneumoniae* has a tiny zone of inhibition of 15 mm at 75 µl dose.[17] In the present study also rGO-Ag nanocomposite obtained from *A. malabarica* was studied for antibacterial activity against the same four pathogenic strains *P. aeruginosa, K. pneumoniae, S. aureus* and *E. coli*. At a dosage of 50 µl, P. aeruginosa showed the highest zone of inhibition.

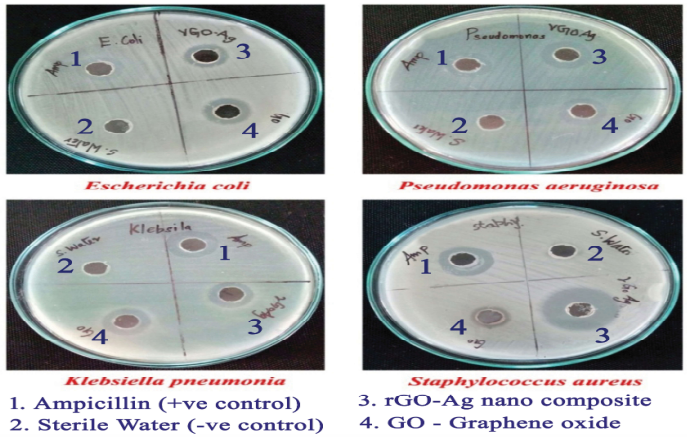

Fig-5: Antibacterial Analysis of GO and rGO-Ag Nanocomposite

Antimicrobial properties of Ag+ and Ag-based composites have been thoroughly investigated.[18] Ag+ can damage bacterial membranes and produce reactive oxygen species (ROS) by photocatalytic activation. However, when Ag nanoparticles engage with bacteria, they clump together, reducing their effective specific surface area and thus their antibacterial activity.[19] Nanocomposites of graphene and Ag nanoparticles were produced to address this issue, while antibacterial efficacy was still restricted.[20-21].As a result, numerous studies on GO and rGO nanocomposites containing Ag nanoparticles have been published, with improved performance compared to GO and Ag nanoparticles alone due to synergistic effects; additionally, the combination of both constituents significantly reduces the concentrations required to inhibit all bacteria. As a result, AgNPs-GO composites with an average Nanoparticles size had considerably stronger antibacterial activity. In this study the synthesized nano composite showed good antibacterial activity and its size may be an average to small in size.

CONCLUSION

This work used a novel green production approach to demonstrate the antibacterial activity of rGO-Ag nanocomposite. RGO-Ag nanocomposite's antibacterial potential was tested against Gram-positive and

Gram-negative bacteria, and the nanocomposite outperformed GO and Ampicillin. Antibacterial applications could benefit from rGO-Ag nanocomposite biomaterials.

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