

UNCOVERING THE MULTIFUNCTIONAL AND TOXICOLOGICAL PROPERTIES OF REDUCED GRAPHENE OXIDE (rGO) NANOSHEETS OVER THE SELECTED BACTERIAL AND FUNGAL SPECIES

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited with Grade "A" by NAAC**

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This is to certify that this Project Report is the bonafide work of A. D. Rakshi (39230035) who carried out the Project entitled "UNCOVERING THE MULTIFUNCTIONAL AND TOXICOLOGICAL PROPERTIES OF REDUCED GRAPHENE OXIDE (rGO) NANOSHEETS OVER THE SELECTED BACTERIAL AND FUNGAL SPECIES" under my supervision from January 2023 to April 2023.

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DECLARATION

I, A. D. Rakshi, hereby declare that the Project Report entitled '**UNCOVERING THE MULTIFUNCTIONAL AND TOXICOLOGICAL PROPERTIES OF REDUCED GRAPHENE OXIDE (rGO) NANOSHEETS OVER THE SELECTED BACTERIAL AND FUNGAL SPECIES**' done by me under the guidance of Dr. S. Jayashree and Dr. Kunal Biswas at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

In recent years, pathogens varying to become resistant to the classic antibiotics are of great concern. The first bacteria to have exhibited this antibiotic resistance is *Staphylococcus aureus*, right from 2007 until today there are not many chemical drugs developed to kill this gram-positive bacterium, due to its resistant modification in the thick peptidoglycan layer. Nanotechnology is a growing field, where it uses nanomaterials for solving many problems in this fast-paced world. One such nanomaterial that has a rough surface according to SEM is graphene, due to which it has properties to disrupt both bacterial and fungal cell walls. Graphene is the most notable substance among the newly found and created nanostructured carbonaceous species. The extraordinary features of graphene (electronic conductivity, mechanical resilience, enormous surface area) have caused a profound shift in the field of material research. In recent years, graphene and its derivative composite materials have divided into various fields of technology. However, varied physicochemical qualities resulted in an extraordinary impact on its biological response. The biocompatibility of this nanomaterial over different higher organisms is a developing research topic. Graphene, having an irregular surface, elicits cidal activity against various organisms depending upon the concentration imparted. With the toxicological investigations of prokaryotes and fungus, graphene family nanoparticles, especially reduced graphene oxide can be developed as an antibiotic against Antimicrobial-resistant bacteria and fungus. This project aims at finding the exact toxic concentration of reduced graphene oxide over bacteria and fungi. The study would also enable us to uncover the toxicity pathway of rGO over bacteria through *in-silico* analysis which will help us devise a potential antibiotic and drug carrier for several targeted drug delivery applications.

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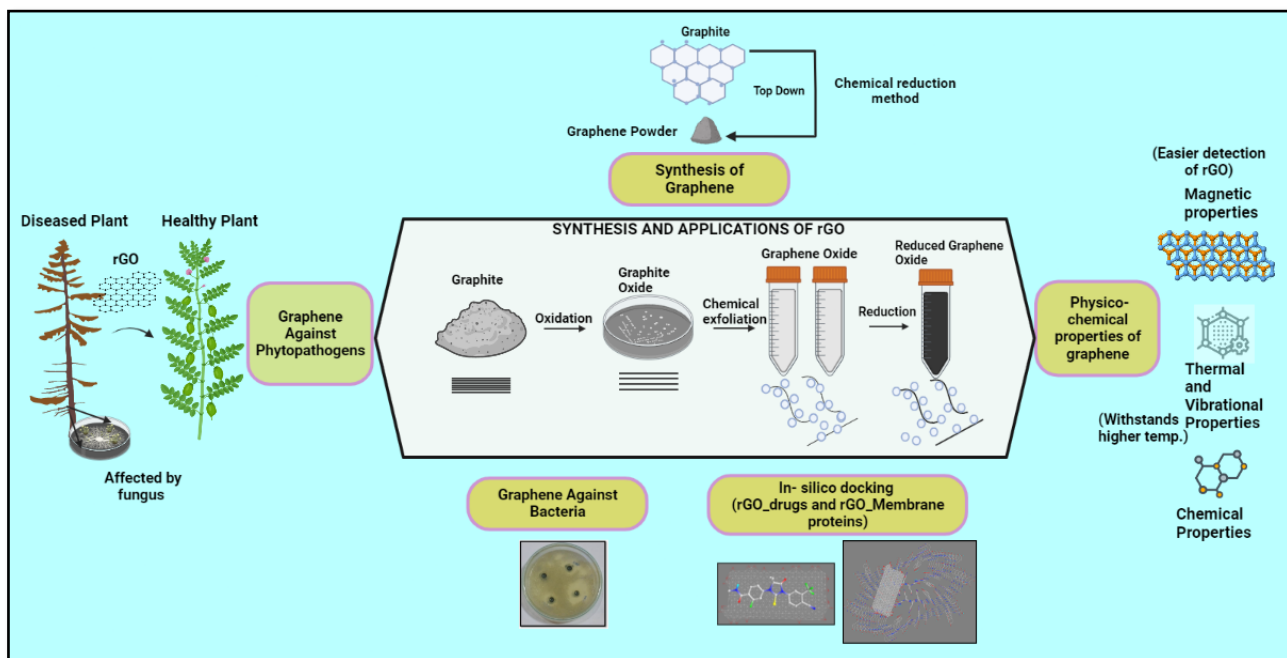
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SCHEMATIC REPRESENTATION OF THE THESIS



List of Abbreviations:

ABBREVIATIONS	FULL FORM
AMR	Antimicrobial Resistance
FOC	<i>Fusarium Oxysporum f.sp. cubense</i>
rGO	Reduced Graphene Oxide
GO	Graphene Oxide
SEM	Scanning Electron Microscopy
EHS	Environmental Health and Safety
GBM	Graphene-Based Materials
GFNs	Graphene Family of Nanoparticles
ZGNR	Zigzag graphene nanoribbon
ROS	Reactive oxygen species
FRET	Fluorescence Resonance Energy Transfer
ssDNA	Single-stranded Deoxyribo Nucleic Acid
2D	Two Dimensional
ATP	Adenosine Triphosphate
HIV	Human Immuno Virus
PEG	Poly Ethylene Glycol
ZOI	Zone of inhibition
MRGO	Modified Reduced Graphene Oxide
OTC	Over the Counter drug

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1. INTRODUCTION

Antimicrobial Resistance (AMR) is the result of bacteria, viruses, fungi, and parasites that have evolved through time and have ceased to respond to antibiotics. This makes infections more difficult to cure and raises the risk of disease transmission, life-threatening sickness, and death. Drug resistance makes it harder or impossible to treat illnesses and renders antibiotics and other antimicrobial medications useless (Marston *et al.*, 2016). In today's fast paced polluted world, fast food has become a common diet to multiple age people. Due to this scenario, lots of youngsters develop infections that are caused by pathogens and as a result, they depend on over-the-counter antibiotics. The fraction of these microorganisms becoming resistant to antibiotics has dramatically increased along with the percentage of antibiotic consumption. Our ability to cure common diseases is still in danger due to the creation and spread of bacteria that are resistant to drugs and have developed new resistance mechanisms. The increasing global development of multi- and pan-resistant bacteria commonly referred to as "superbugs," which cause diseases that cannot be treated with currently available antimicrobial medications like antibiotics, is particularly concerning (Marston *et al.*, 2016).

There are no novel antimicrobials in the clinical pipeline. Only six of the 32 antibiotics that address the WHO list of priority infections that were listed by WHO as being in clinical development in 2019 were considered novel. Access to high-quality antimicrobials also continues to be a big problem. All levels of development are being impacted by antibiotic shortages, particularly in healthcare systems. AMR has a significant financial impact on national economies and health systems because it reduces patient or caregiver productivity by necessitating longer hospital stays and more expensive, intensive care (Hofer, 2019). The first bacteria to develop resistance over penicillin and methicillin antibiotics is *Staphylococcus aureus* (Mukherjee *et al.*, 2021), the graph of this data is illustrated in *Figure 1.1*. After which *Escherichia coli* and *Proteus* also developed resistance. In this project, the anti-bacterial assay is done for all the above-listed three bacteria and the rGO nanosheets are toxic to them at 400µg/ml concentration. rGO nanosheets are also incorporated with several fungal

phytopathogens that affect crop production are also used, such as *Pyricularia oryzae*, *Fusarium oxysporum* f.sp. *cubense*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Nigrospora oryzae*, and *Athelia rolfsii* with which anti-fungal assay is performed. One of them is *Fusarium oxysporum* f.sp. *cubense* (Foc) that affects the banana plant globally. This global distribution of Foc has been depicted in Figure 1.2. This project aims at understanding the interaction and the precise toxic concentration of rGO nanosheets over various fungal phytopathogens that are tabulated in Table 1.1. As this is a global concern, evolving a solution for this problem has become an important field of research. Nanotechnology, being an emerging and promising field for medical issues has led a path for developing an antibiotic for resistant bacteria.

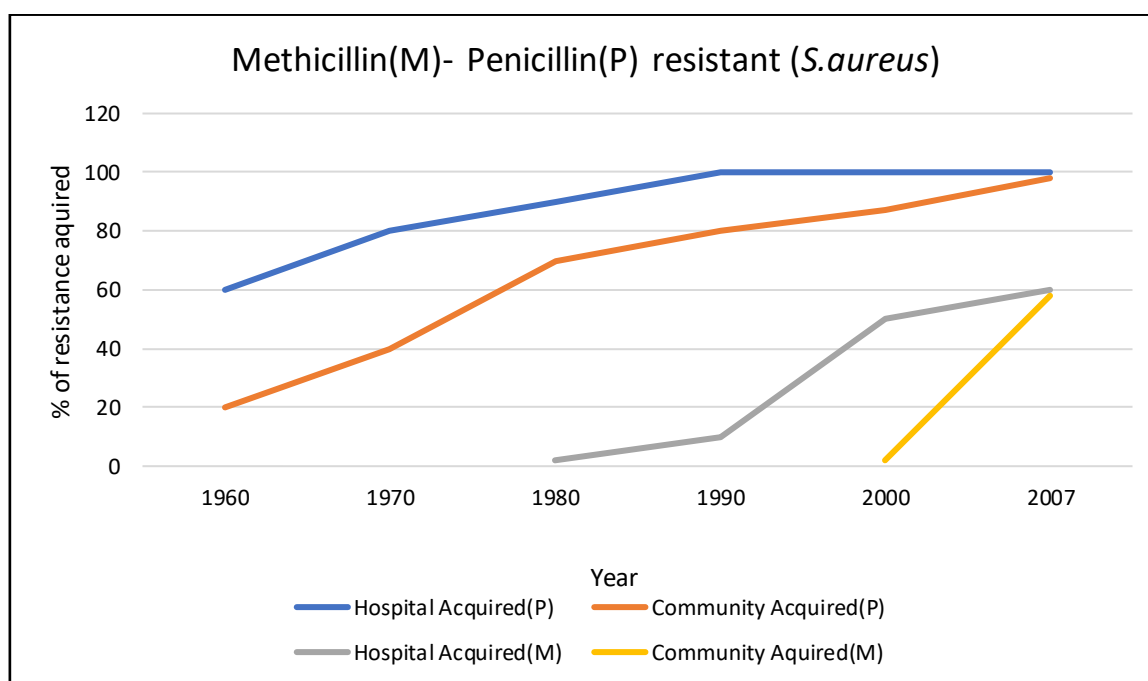


Figure 1.1: Graph showing the evolution of AMR in *S.aureus*

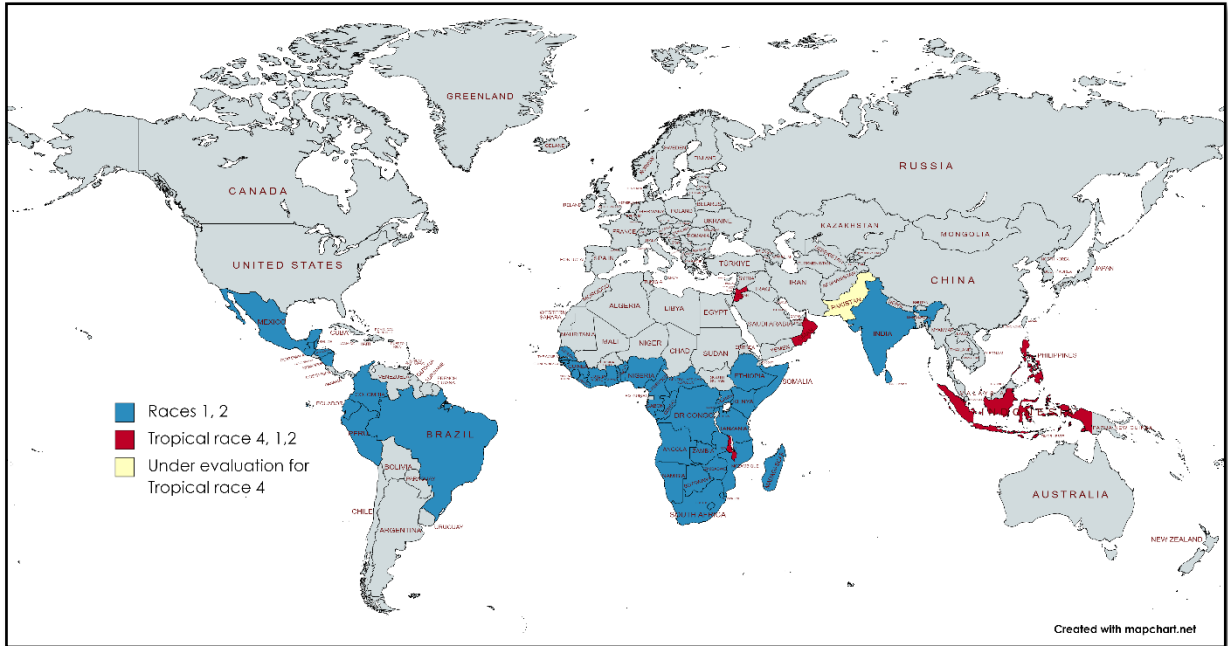


Figure 1.2: Global distributions of races of *Fusarium oxysporum* f.sp. cubense (Foc), causative agent of *Fusarium* wilt of banana

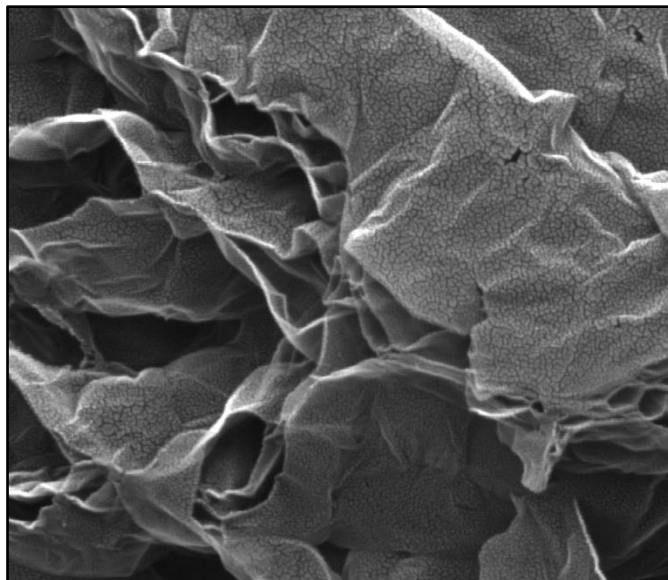
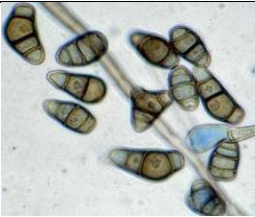
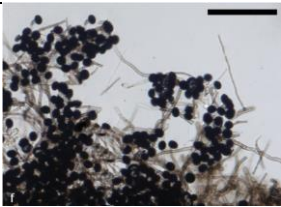
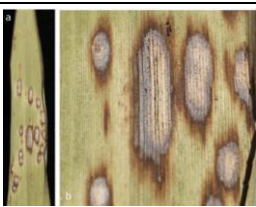


Figure 1.3: SEM image of rGO nanosheets

Table 1.1: Fungal phytopathogens and the disease caused

PHYTO -PATHOGENS	MICROSCOPIC IMAGE	DISEASE CAUSED	SYMPTOMS IMAGE	REFERENCE
<i>Pyricularia oryzae</i>		Rice blast		(TNAU Agritech Portal :: Crop Protection, n.d.)
<i>Fusarium oxysporum</i> f.sp. <i>cubense</i>		<i>Fusarium</i> wilt		(Jamil et al., 2019)
<i>Colletotrichum gloeosporioides</i>		anthracnose		(How to Identify, Treat, and Prevent Anthracnose, n.d.)
<i>Curvularia lunata</i>		Turf		(<i>Curvularia</i> Leaf Spot Turf Disease Syngenta Turf and Landscape, n.d.)
<i>Nigrospora oryzae</i>		White mould		(Lianmeng Liu et al., 2021)
<i>Athelia rolfsii</i>		Southern blight		(Elahi et al., 2021)

Graphene is extremely conductive, and flexible, and has adjustable permittivity and hydrophilicity properties (Geim, 2009; K.S.Novoselov, 2011). Especially, reduced graphene oxide (rGO) has a rough surface area, which disrupts the cell wall of the bacteria and fungus and elicits the apoptosis mechanism over them. The SEM image of reduced graphene oxide is depicted in *Figure 1.3*. These characteristics pave the way for the creation of multipurpose biomedical devices and antibiotics or drug carriers (Konstantin S Novoselov *et al.*, 2012). Evaluation of binding affinities of graphene with components of the biological environment to ascertain the possibilities and constraints provided is a highly important and significant topic for such implementations. Graphene biological studies should be carried out using highly specialized, carefully constructed, and described materials under predetermined exposure. Nanomaterials linked to graphene have now been created in a variety of sizes, shapes, and surface modifications, giving them a range of different physical, chemical, and biological capabilities.

In-vitro, ecotoxicological investigations and thorough evaluations of the biocompatibility of graphene-related materials before *in vivo* application are essential to produce graphene-related biomedical products. The study of the biological effects of the graphene family of nanomaterials is rapidly growing, with new areas of inquiry focused mostly on medical implications as well as Environmental Health and Safety (EHS). Like any other nanomaterial, the possibility of toxicity raises questions in nonbiomedical sectors where unanticipated occupational, consumer, and environmental exposures might arise (Kostarelos and Novoselov, 2014). Since 2010, several studies and analyses on the nanotoxicology and biocompatibility of materials connected to graphene have been published (Kiew *et al.*, 2016; Tonelli *et al.*, 2015). The literature on the graphene-biological interface is evaluated in this study, with an emphasis on basic biological reactions and processes that are crucial for both practical applications and material safety. It is emphasized throughout the project that variations in the potential biological effects may lead to a variety of biological responses that, if understood, can be wisely used for function or security reasons (Sanchez *et al.*, 2012).

2. REVIEW OF LITERATURE

2.1 HISTORY OF GRAPHENE NANOSHEETS

Andre Geim and Konstantin Novoselov, two researchers at the University of Manchester in the United Kingdom, discovered graphene in 2004, making it a relatively new substance in the realm of materials science (Miller *et al.*, 2018). Yet, since the 1940s, the concept of a two-dimensional substance made exclusively of carbon atoms organized in a hexagonal lattice has been proposed. The discovery of graphene was made possible by the use of a simple procedure called mechanical exfoliation, in which a layer of graphite was repeatedly scraped away using sticky tape until just a single layer remained (Meyer and Hviid, 2013). Scanning tunneling microscopy was then used to study this single layer of graphene, confirming its special characteristics. Due to its remarkable mechanical, thermal, and electrical characteristics, graphene has subsequently become a hotly debated scientific topic. Being one of the most conductive materials and the strongest substance yet known, it is often regarded as both. Its special mix of attributes makes it a potentially valuable material for a variety of applications, including electronics and energy storage as well as biomedical devices and sensors. Geim and Novoselov received the 2010 Nobel Prize in Physics for their revolutionary work on graphene (Wolf, 2014). Since that time, graphene research has expanded, and new applications and prospective uses are consistently found.

2.1.1 *Reduced graphene*

Reduced graphene is a kind of graphene that has undergone a reduction procedure to remove part or all of the oxygen functional groups that are commonly present on the surface of graphene oxide (Thakur and Kandasubramanian, 2019). This technique results in a material that is more comparable in characteristics to pure graphene. A research team lead by Rodney Ruoff at the University of Texas at Austin announced the first reduction of graphene oxide in 2006. They employed hydrazine as a reducing agent to remove the oxygen functional groups from graphene oxide, yielding

reduced graphene oxide (rGO). Since then, the manufacture of rGO has been a focus of significant study, with numerous techniques for reducing graphene oxide and producing rGO with varying characteristics being discovered. Chemical reduction, thermal reduction, and electrochemical reduction are some of these approaches (Olabi *et al.*, 2021). Reduced graphene oxide possesses features comparable to graphenes, such as excellent electrical conductivity and mechanical strength, but with the additional benefit of being simpler to produce and process (Zhu *et al.*, 2010). It is a promising material for a variety of applications, including energy storage, catalysis, and sensing. Overall, the invention of reduced graphene oxide has created new opportunities for investigation and prospective applications in the area of materials science.

2.1.2 Graphene oxide

A graphene derivative with surface-attached oxygen functional groups is called graphene oxide. By processing graphite with a solution of potassium chlorate and sulfuric acid, Benjamin Brodie created the first synthetic version of it in 1859 (Brisebois and Siaj, 2020). This substance was known as graphite oxide. Unfortunately, it took a long time for the genuine single-layer nature of graphene oxide to be understood. When Andre Geim and Konstantin Novoselov and their colleagues at the University of Manchester discovered the extraordinary characteristics of graphene in 2003, interest in graphite oxide and its derivatives was rekindled (Neto and Novoselov, 2011). The first effective mechanical exfoliation of graphene from graphite was announced in 2004 by a different team of researchers at the University of Manchester, headed by Geim and Novoselov (Tiwari *et al.*, 2018). They did, however, also point out that the exfoliation procedure often resulted in graphene oxide flakes, which they referred to as a "dirty version of graphene." Since then, several techniques have been developed to synthesis, analyze, and use graphene oxide, which has been a focus of significant study. It is often used as a starting material for the creation of reduced graphene oxide, a material with characteristics closer to those of pure graphene (Jiříčková *et al.*, 2022). Many possible uses for graphene oxide exist, including healthcare, energy storage,

and electronics. It is a potential material for a variety of applications because of its distinctive qualities, including its large surface area, water dispersibility, and customizable functional groups (D. Chen *et al.*, 2012).

2.1.3 Reduced graphene oxide

Reduced graphene oxide (rGO) is a kind of graphene oxide that has had its surface oxygen functional groups partially or completely removed by a reduction process (Chowdhury *et al.*, 2015). A material with characteristics that are more akin to pure graphene is produced by this technique. Graphene oxide was successfully reduced for the first time in 2006, according to research done at the University of Texas at Austin under the direction of Rodney Ruoff (Warner *et al.*, 2012). They created rGO by stripping the oxygen functional groups from graphene oxide using the reducing chemical hydrazine. Since then, other approaches to reducing graphene oxide have been created, including chemical reduction, thermal reduction, and electrochemical reduction (Sharma *et al.*, 2017). Several techniques have been utilized to create rGO with different characteristics and levels of oxygen removal. High electrical conductivity and strong mechanical strength are two characteristics of reduced graphene oxide that are comparable to those of graphene, with the additional benefit of being simpler to produce and treat. It is a promising substance for several uses, such as energy storage, catalysis, and sensing. Overall, the creation of reduced graphene oxide has increased the possible uses of graphene-based materials in a variety of disciplines and created new prospects for the manufacturing of customized graphene-like materials (Jindal *et al.*, 2022).

2.2 SYNTHESIS OF GRAPHITE TO GRAPHENE

2.2.1 Physical method

There are several physical methods for the synthesis of graphene from graphite, including:

2.2.1.1 Mechanical exfoliation

Using scotch tape or another sticky tape, single-layer graphene is separated from bulk graphite in this process (Lin *et al.*, 2011). To remove a thin coating of graphite that may subsequently be further processed to produce graphene, the tape is applied to the graphite surface and pulled off.

2.2.1.2 Chemical Vapor Deposition (CVD)

This process involves introducing a gaseous precursor that contains carbon into a hot furnace, where it decomposes and deposits a layer of graphene on a substrate. The substrate may be built of various materials, such as copper or nickel, and the reaction conditions can be changed to regulate the graphene's thickness and quality.

2.2.1.3 Plasma-enhanced chemical vapor deposition (PECVD)

A plasma is employed in this CVD version to speed up chemical processes and increase the quality and uniformity of the graphene layer (Sun *et al.*, 2015).

2.2.1.4 Epitaxial growth

In this process, graphene is grown on a material, such as silicon carbide, that has a crystal structure that is comparable to that of graphene. Epitaxy is the process by which the graphene atoms align with the substrate lattice to produce the graphene layer on top of the substrate (Mishra *et al.*, 2016).

These are just a few examples of physical processes used to create graphene from graphite. The best way to choose will depend on the particular application and needs since each method has benefits and limits.

2.2.2 Chemical method

Various chemical processes may be used to create graphene from graphite, such as:

2.2.2.1 Hummer's method

In this procedure, concentrated sulfuric acid, potassium permanganate, and sodium nitrate are used to oxidize graphite and form graphene oxide (GO) (Hou *et al.*,

2020). A reducing agent like hydrazine may be used to further decrease GO to produce reduced graphene oxide (rGO).

2.2.2.2 Chemical reduction of graphene oxide

By applying a reducing agent such as sodium borohydride, ascorbic acid, or hydroquinone, GO may be chemically reduced to generate rGO. Graphene is created when the sp^2 carbon-carbon bonds that were broken during the oxidation process are repaired during the reduction step.

2.2.2.3 Solvothermal method

With this technique, graphite is mixed with a solvent, such as N-methyl pyrrolidone (NMP), and then heated to high pressures. Exfoliation and reduction of graphite occur as a result of the process, of producing graphene (Zhang *et al.*, 2018).

2.2.2.4 Electrochemical exfoliation

In this technique, graphite is exposed to an electric field while submerged in an electrolyte solution. Graphene is created when graphite exfoliates due to an electrochemical process.

2.2.3 Biological method

The development and research of biological processes for the synthesis of graphene from graphite are still in their infancy. Yet, recent research has shown that certain microbes, including bacteria and fungi, can create enzymes that may reduce graphene oxide (GO) to graphene. These are a few instances of biological processes used to create graphene:

2.2.3.1 Bacterial reduction

It has been reported that GO can be reduced to graphene using a variety of bacteria, including *Shewanella* and *Escherichia coli*. Reductases are one kind of enzyme that the bacteria make that can reduce the oxygenated functional groups on GO and restore part of the sp^2 carbon-carbon bonds to create graphene.

2.2.3.2 Fungal reduction

It has also been shown that certain fungi, like *Pleurotus ostreatus* and *Trametes versicolor*, may create enzymes that can reduce GO to graphene (H. Yang *et al.*, 2018). Laccases and peroxidases, two enzymes that may catalyze the reduction of the oxygenated functional groups on GO, are engaged in this process.

2.2.3.3 Plant-mediation reduction

It has been shown that several plant extracts, including grape and green tea extracts, have reducing characteristics that can convert GO to graphene. Several different antioxidants and polyphenols found in plant extracts may function as reducing agents and help to repair some of the sp^2 carbon-carbon bonds on GO.

Traditional chemical techniques for the manufacture of graphene may not be as ecologically benign or as sustainable as biological approaches. To enhance the circumstances and increase the effectiveness of these techniques, further study is required.

2.3 PHYSICO-CHEMICAL PROPERTIES OF 2D GRAPHENE SHEETS

2.3.1 Surface properties of graphene

The degree of the disruption in the potential or breakdown of the underlying sublattice symmetries has a significant impact on the primary scattering processes and related transport properties of graphene (Ritter *et al.*, 2009). Short-range interactions in graphene may disclose a broad variety of physical processes since all intravalley and intervalley scattering events between K and K' sites are allowed. Strong backscattering events and localization effects are caused by chirality breaking, which can be induced by short-range potentials created by atomically sharp defects like vacancies, Anderson disorder, or edge deformations (Rosales *et al.*, 2009). In particular, graphene nanoribbons are inherently prone to edge disorder due to the greater reactivity of borders that may be sensitive to chemical passivation, irregularity, and structural restoration (Cresti and Roche, 2009). The noteworthy physicochemical properties of graphene oxide includes its huge surface area, ease of surface

functionalization, and increased colloidal stability in aqueous conditions (Bitounis *et al.*, 2013). But as graphene-based materials (GBMs) are produced and used more, it's important to carefully consider how they affect cells and tissues. It is very crucial to comprehend how immune system interactions work (Farrera and Fadeel, 2015). Neutrophils, macrophages, and dendritic cells (DCs), phagocytic cells of the innate immune system, will come into touch with a foreign material that has entered the body. In addition to serving as the body's primary line of protection against foreign invaders, these cells also remove dead cells, which is essential for preserving tissue homeostasis. *Figure 2.1* lists the chemical and physical characteristics of graphene.

2.3.1.1 *Vibrational and Thermal properties*

At ambient temperature, individual graphene thermal conductivity is mostly attributed to acoustic phonons. Umklapp processes are repressed when the number of layers is decreased, and the big value, which relates to lengthy entail pathways of phonons, is explained (Ghosh *et al.*, 2010). The quadratic dispersion of the acoustic flexural mode ZA in two-dimensional crystals is near the Brillouin zone point, in addition, it displays uniqueness in the density of states at zero energy. Low-dimensional crystals should be unstable because thermal fluctuations are predicted to cause atomic displacements that are as great as the interatomic distance at limited temperatures. It is shown that macroscopic graphene samples, on the other hand, maintain their crystal quality and are stable, which is assumed to be brought on by the existence of minute crumpling in the third dimension. Graphene has an isotropic thermal conductivity in ballistic motion. Below 20K, the ZA mode prevents conduction from happening. Although the ZA mode predominates in terms of thermal conduction, the LA and TA modes are also present above 20K. The exact solution of the phonon Boltzmann equation indicates that the ZA mode is also the major heat transporter at higher temperatures.

When immune cells or other biomolecules in the biological milieu were exposed to GBNs, their fundamental qualities underwent a dynamic shift that might result in biotransformation or annihilation. Therefore, *in-situ* assessments are essential for future applications (Jiménez and Tongeren, 2017). Several taxa, including bacteria,

plants, invertebrates, and vertebrates, as well as their primary target organs, including the cardiovascular and immune systems, have been examined in recent research to determine the toxicity of GBNs. There is evidence that GBNs may cross physiological barriers and enter distant secondary organs after an initial invasion (Fadeel *et al.*, 2018).

2.3.1.2 *Mechanical properties*

We were the first to directly assess the mechanical properties of monolayer graphene AFM by nano indenter-suspended monolayer graphene membranes using an atomic force microscope. Graphene was awarded the moniker "strongest material ever" after the verification of its lasting breaking strengths with an inherent mechanical strain and Young's modulus (Lee *et al.*, 2008). It is also feasible to modify the mechanical thickness of the substance, as shown by machine-driven pressure tests executed on graphene sheets exposed to deflections brought on by applying diverse insulating capping layers (Ni *et al.*, 2008). The experimental findings about the fundamental mechanical characteristics of graphene have been validated by theoretical analyses using a range of techniques. Several techniques, such as *ab initio* (F. Liu *et al.*, 2007), tight binding (Pereira *et al.*, 2009), molecular dynamics simulations, and semiempirical models, have been used to precisely quantify graphene's Young's modulus and other inherent mechanical characteristics (Scarpa *et al.*, 2009). Due to the possibility of using these thin, strong, and adaptable materials to make structural elements for nanoelectromechanical systems (NEMS), graphene's exceptional mechanical qualities have also piqued the curiosity of electronic applications. For the creation of affordable NEMS devices, a flawless match between the machine-driven and electric responses of the conductive channel is required. In this respect, the efficiency of a graphene-based NEMS is solely dependent on the viability of performing band gap engineering using external mechanical forces (Q. Lu and Huang, 2010).

2.3.1.3 *Magnetic properties*

Several theoretical research based on density functional theory or the mean-field Hubbard model predicts that the edge states of graphene would become spin-polarized

owing to a magnetic instability generated by the corresponding spike in the density of states only at Fermi energy (EF) (Yazyev, 2010). The atoms are antiferromagnetically arranged across opposing edges in the electromagnetic ground state when an energy gap at EF is formed and ferromagnetically directed along one edge. This antiferromagnetic ground state is in agreement with Lieb's theorem for a bi-particle lattice in a Hubbard model description. Edge magnetism is a phenomenon that is thought to occur in all graphene systems with zigzag edge segments, not just perfect ZGNRs (Cantele *et al.*, 2009). Building a magneto resistor exploiting the edge magnetism phenomena is an area of potential application (Muñoz-Rojas *et al.*, 2009). However, there hasn't been any experimental proof of graphene edge magnetism to date (Sepioni *et al.*, 2010). Only an indirect observation of magnetic edges using local probe microscopy has been made (Joly *et al.*, 2010). Theoretical works contend that the ideal zigzag-edged appearance is not very likely to occur, which supports the lack of magnetic edge states observed. Some nonmagnetic edge topologies have substantially better thermodynamics (Kunstmann *et al.*, 2011). The antiferromagnetic ground state was also shown to be only stable in perfect ZGNRs at freezing temperatures. Increasing amounts of evidence thus support the lack of edge magnetism as a true occurrence at ambient temperature.

2.3.1.4 *Elastic properties*

The nonlinear elastic characteristics of graphene, which have a second-order elastic toughness of -690 nm in the non-linear domain and a 2D Young's modulus of 340 nm, were assumed to be the reason for the indentation force-displacement connection. Using the finite element technique FEM and an anisotropic, singly perturbed elastic model for graphene as predicted by density functional theory DFT simulations, Wei and Kysar (Wei and Kysar, 2012) conducted a more thorough study of the nanoindentation experiment. The mechanical properties of polycrystalline graphene films made by chemical vapor deposition (CVD) with varied grain sizes were then investigated using AFM indentation investigations. Further investigation revealed that the stiffness of graphene in a plane would be greatly reduced by the presence of out-of-plane ripples (Ruiz-Vargas *et al.*, 2011). The nonlocal elasticity theory considers the effects of

nanostructures on very small-length scales, such as nanoplates. Visual representations are provided of the smallscale or nonlocal impacts on the non-dimensional buckling loads in the presence of aspect ratio and buckling modes (Tourki Samaei and Hosseini Hashemi, 2012).

2.3.1.5 *Non-Elastic properties*

Despite elasticity, flaws' presence and how they evolve during redirection have a substantial impact on the mechanical properties of 2D materials. In terms of stress and energy, respectively, endurance and robustness are two physically separate qualities that explain the onset of failure. In fundamental mechanics, the energy per unit volume of the material, expressed in J/m^3 , is used to determine hardness. The energy content of an area per unit of crack propagation is expressed using the unit J/m^2 , which is more properly defined by fracture mechanics. When using a unit of J/m or eV/nm instead of the 2D Young's modulus (unit: N/m), the fracture toughness of 2D materials may be expressed as the energy/length of crack propagation (S. Singh and Patel, 2014). This section reviews the many types of faults in two-dimensional materials, their strength and toughness, and potential toughening methods.

2.3.1.6 *Strength and Toughness*

Graphene has been identified as the strongest material now in use as a typical 2D material, with a long-lasting durability of 130 GPa. Even though computations at the atomic level have been done to attempt to explain the relationship between roughness and particle size in polycrystalline graphene, the tests mentioned have not even shown a qualitative consistency (Mohan *et al.*, 2018). From an experimental perspective, it is not always possible to determine the weakest triple junction or grain boundary that controls a sample's failure strength using the widely utilized AFM nano-indentation technique. It has been shown that the failure strength for polycrystalline graphene with randomly scattered grains exhibits significant statistical changes following a Weibull distribution, as shown by Molecular Dynamics (MD) simulations and theoretical modeling based on the "weakest-link" statistics.

2.3.2 Electrical and electronic properties of graphene

Graphene is a hexagonal lattice made up of a single layer of carbon atoms. Four valence electrons and the electronic shell configuration [He] $2s^2 2p^2$ are present in a single carbon atom in its ground state. Hybridized orbitals, which are created when 2s are overlapped with $2p_x$ and $2p_y$ orbitals, are what give rise to the carbon-carbon covalent bond in graphene (Lizhao Liu *et al.*, 2016). The hexagonal arrangement efficiently confines and stabilizes the three neighboring carbon atom bonds created by the planar orbitals, which are accountable for the bulk of the binding energy and the elastic properties of the graphene sheet. The intersection of these orbital states between neighboring atoms greatly influences the electrical properties of graphene. The remaining independent $2p_z$ orbitals have Π symmetry orientation (Yuan *et al.*, 2015). As a result of its electronic states is easily represented by a linear combination of $2p_z$ orbitals, graphene's electrical structure may be accurately approximated by an orthogonal proximity tight-binding approximation. The energy dispersion relation of the Π (bonding) and $^*\Pi$ (antibonding) bands may be obtained by solving the Schrödinger equation, which is reduced to a matrix secular expression (Dubois *et al.*, 2009). The extraordinary electrical qualities of graphene have propelled researchers to investigate their fundamental physical characteristics with the added benefit of converting them into useful applications. Nevertheless, its use in digital devices is severely constrained by the absence of an energy-band gap. Therefore, scientists are seeking fresh methods to make graphene develop a band gap.

An antibody-antigen immunological reaction changes the electronic state of the graphene surface, which may be seen, for example, by a change in the current flowing through a graphene chip (Huang *et al.*, 2020). The properties of graphene as well as the physical and chemical processes that occur on the graphene surface during the whole biosensor manufacturing process have an impact on the ultimate sensitivity of such a device (Beitollahi *et al.*, 2019). It is also affected by how uniform these activities are over the whole graphene chip. The bulk of the focus has been on functionalizing graphene and creating contact pads that ensure virus detection on a chip's graphene surface (Lebedev *et al.*, 2021).

2.3.3 Opto-electronic properties of graphene sheets and applications

Graphene has extraordinary optical qualities, in addition to the burgeoning interest in its electrical properties. One of graphene's most well-known optical features is its ability to operate as a powerful quencher for a variety of luminous dyes and nanoparticles (Chang and Baek, 2016). Due to two opposing mechanisms—photoinduced electron transfer and intramolecular energy transfer facilitated by a through-bond mechanism because of the luminophore's covalent attachment—this is the case (Terrones *et al.*, 2011). The wide spectrum luminescence and high optical transmittance of graphene, on the other hand, are outstanding optical qualities.

2.3.4 Tribological properties of graphene sheets

The effectiveness of micro-electromechanical systems (MEMS), for example, which is extremely precise mechanical systems and nano-electro-mechanical systems (NEMS), as well as metal matrix composites and boundary lubrication, depends greatly on the tribological behavior of the materials. These mechanical failures are often caused by friction and wear in moving sections (Kumar and Wani, 2017). The efficiency of parts like gears, switches, and actuators is significantly influenced by their resistive conductance because NEMS and MEMS components are small and have tight tolerances, whereas the component's mechanical and commercial viability is determined by its wear and tear resistance (Hae Jin Kim *et al.*, 2012). However, standard techniques for lubricating fluids to minimize friction cannot be used in microsystems owing to the effects of surface tension. To build reliable MEMS and NEMS devices, it is crucial to enhance the tribological properties of the parts/components under dry sliding conditions. This is accomplished by organic substances such as bi-layer and multi-layer hard coatings (Penkov *et al.*, 2013), self-assembled monolayers (H. J. Kim *et al.*, 2009), diamond-like carbon (DLC) films (Penkov *et al.*, 2012), and nanostructured coating. One such revolutionary material that has shown considerable potential in terms of tribological characteristics is graphene.

Over the last ten years, the world of materials has been more interested in graphene due to its distinct properties, such as strength, hardness, and fracture toughness. Numerous unique physical, mechanical, and electrical features of graphene have been extensively studied (Peng *et al.*, 2008). Additionally, graphitic carbon exhibits a flawless crystallographic sheet. For improved lubricity and wear resistance, graphene platelets have been used as oil additives (Lin *et al.*, 2011). More significantly, graphene works well as a corrosion protective layer on refined metals like copper and nickel when utilized in a reactive atmosphere for mechanical purposes (Prasai *et al.*, 2012). Earlier tribological research focused on graphite flakes revealed that graphite scrolls develop at the tribological contact. These scrolls are excellent for lowering friction at sliding surfaces and decreasing surface energy. In light of their exceptional chemical, mechanical, thermal, and electrical properties as well as their comparatively exceptionally low manufacturing costs, graphene sheets stand out from other materials used in tribological applications, as shown in *Figure 2.2*. As a result, graphene is seen as a potential material for use in NEMS, MEMS, and other tribological applications in the future. Graphene also offers a great deal of promise for application as an ultra-thin protective covering and as a self-lubricating material for a variety of precision components that are exposed the contact stress because of its greater strength (Skoda *et al.*, 2014). This property can be further developed for producing nano-bio sensors, which would use biological systems as the input.

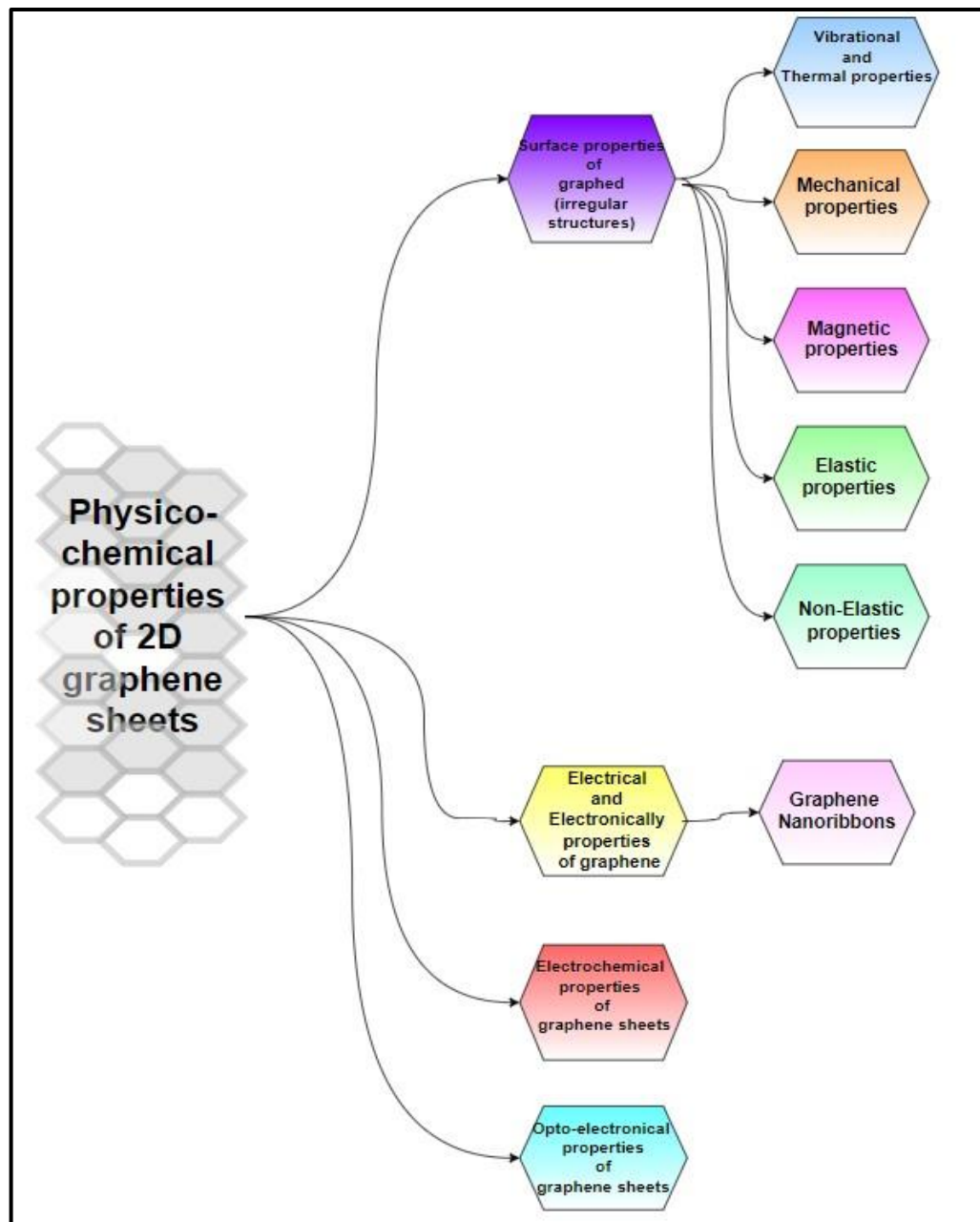


Figure 2.1: Physical and chemical characteristics of 2D graphene sheets

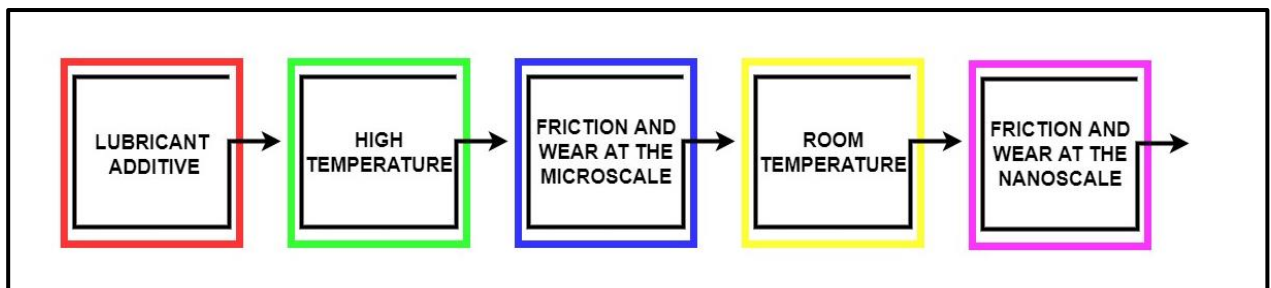


Figure 2.2: Basic Tribological properties of graphene sheets

2.4 TOXICOLOGICAL STUDIES OF 2D GRAPHENE SHEETS IN BIOLOGICAL SYSTEM

2.4.1 Activity of graphene against prokaryotes

GO sheets are proven to have antibacterial properties (Akhavan and Ghaderi, 2010a) and are presently being researched for use as antibacterial materials (such as in bandages). It also inhibits cell growth at sublethal concentrations which are caused due to extracellular deficiency (Biswas *et al.*, 2021).

Compared to other materials like minerals, metals, and polymers, very little research has examined how bacteria interact with graphene. As graphene has an unusual surface which is in particular because of its high aspect ratio physical dimension, it is possible to design surfaces with specific qualities that might prevent or encourage bacterial adhesion as well as perhaps trigger cell death. The capacity to tailor the interaction with bacteria is made possible by various preparation techniques that create single-layer and multi-layer graphene sheets (Jastrzębska *et al.*, 2012). While some research has shown that these surfaces are anti-bacterial, other research has demonstrated that they promote cell adherence. In 2010, (Akhavan and Ghaderi, 2010b) first carried out research to investigate how bacteria interact with a graphene-like surface using Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. In this evaluation, edges of reduced graphene oxide, single-layer graphene oxide, and few-layer graphene oxide were placed on a stainless-steel substrate. Both *E. coli* and *S. aureus* had considerably lower cell viability. *Escherichia coli* and *Salmonella typhimurium*, two gram-negative bacteria, and *Enterococcus durans*, *Staphylococcus aureus*, and *Bacillus subtilis*, three gram-positive bacteria, were used to test the antibacterial activity of the synthesized pyrazoles. When tested against the *S. aureus* strain, all of the synthesized pyrazoles showed rather significant levels of antibacterial activity, while none did the same for *E. coli* (Khodadad *et al.*, 2021). Using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) techniques, the antibacterial activity of the items was examined (Amininia *et al.*, 2019). Gram-positive bacteria exhibited decreased cell viability than gram-negative ones (Biswas *et al.*, 2018). The scientists also suggested that reduced graphene oxide

particles would be more potent since they would have sharper edges than their oxidized counterparts. The mechanism behind graphene's ability to kill bacteria is that it seems to trigger membrane rupture when its exposed sharp edges and thinned-out graphene oxide sheets come into contact with bacteria. GO scattering demonstrated the maximum antibacterial activity, with 89.7% of viability loss at 40 mg mL⁻¹, as reported by Liu (S. Liu *et al.*, 2011), in their analysis of the antibacterial properties of four graphene-based materials against *Escherichia coli*, graphite (Gt), graphite oxide (GtO), GO, and reduced graphene oxide (rGO) (*E. coli*).

The research by Ruiz (Ruiz *et al.*, 2011) was contradictory to the studies above, they demonstrated that bacteria are not adversely affected and noted that bacterial growth rates were higher. These surfaces were made on graphene oxide paper. The first bacteria to develop antibiotic resistance is *Staphylococcus aureus*, whose resistance was developed against Penicillin and Methicillin from the year 1940 to the year 2007 and the targets where the resistance was developed is illustrated in *Figure 2.3* (Ahmad, 2018). And right from 2007 to till date, there is no proper drug to kill the antibiotic resistance developed *S. aureus*.

2.4.1.1 Mechanism of interaction

In addition to deterring bacterial adhesion, GMs with antibacterial properties may directly kill or starve prokaryotic cells. It is believed that GM bactericidal action is significantly influenced by membrane stress, oxidative stress, and wrapping isolation (Hegab *et al.*, 2016; Zou *et al.*, 2016). GMs may produce reactive oxygen species (ROS) such as superoxide radicals(O₂⁻), hydroxyl radicals (OH⁻), and hydrogen peroxide when microbes are present (H₂O₂) (Perreault *et al.*, 2015). By entering the membrane and eliminating the phospholipids, GMs kill bacteria (Tu *et al.*, 2013). When proteins, membrane lipids, and bacterial nucleic acids are under oxidative stress, they oxidize and disintegrate, which kills the cells. When exposed to GMs that produce ROS, the cell membrane becomes disorganized due to a significant event called lipid peroxidation (Krishnamoorthy *et al.*, 2012). The complete mechanism is depicted in *Figure 2.4*. Bacterial cells are wrapped in GM sheets to keep them isolated from their growing media, a process known as wrapping isolation (Akhavan *et al.*, 2011). Both Gr

and GO nanosheets have been seen to work via this method, which stops nutrients from passing through cell membranes and causes growth restraint.

According to several kinds of research, GM toxicity varies depending on the dosage. For example, (Gurunathan *et al.*, 2012) revealed that at dosages less than 25 mg L⁻¹, neither GO nor rGO influenced the survival of the Gram-negative opportunistic bacterium *Pseudomonas aeruginosa*. The viability decreased by more than 50% when the concentration of GO or rGO was raised to 100 mg L⁻¹. When 25 mg of L⁻¹ GO or rGO is added, similar outcomes have been found for the model bacteria *E. coli* with no reduction in cell viability. Viability decreased with a 100 mg L⁻¹ increase in GM concentration. *E. coli* cultivation needs to be at or less than 50%. Song (C. Song *et al.*, 2017) evaluated the growth of the biofilm.

2.4.1.2 Membranous proteins interacting with rGO

The interaction of bacterial membrane proteins with reduced graphene oxide (rGO), a type of graphene oxide that has been processed to remove part or all of its oxygen functional groups, has been the subject of several research. Some of the key conclusions are:

2.4.1.2.1 Antibacterial activity

Research has shown that rGO can inhibit the growth of several bacteria, including *E. coli*, *P. aeruginosa*, and *S. aureus*. It is thought that this activity results from rGO's contact with the bacterial cell membrane, which may cause harm and cell lysis (Priya Swetha *et al.*, 2018).

2.4.1.2.2 Adsorption of proteins

It has been shown that rGO may absorb proteins, including membrane proteins, from bacterial cell membranes. The bacterial cell membrane may alter as a result of this adsorption, which may also have an impact on the survivability of the bacteria.

2.4.1.2.3 Modification of bacterial cell membrane properties

It has been hypothesized that rGO may alter the fluidity and permeability of bacterial cell membranes. This may alter how membrane proteins operate and alter the physiology of bacteria.

In general, the relationship between bacterial membrane proteins and rGO is a complicated topic that is continuously being investigated. It is still unclear exactly how the interactions work and how they affect the physiology and survival of bacterial cells. Nonetheless, these investigations imply that rGO may be useful as an antibacterial drug or as a tool for researching the relationships between bacterial membrane proteins.

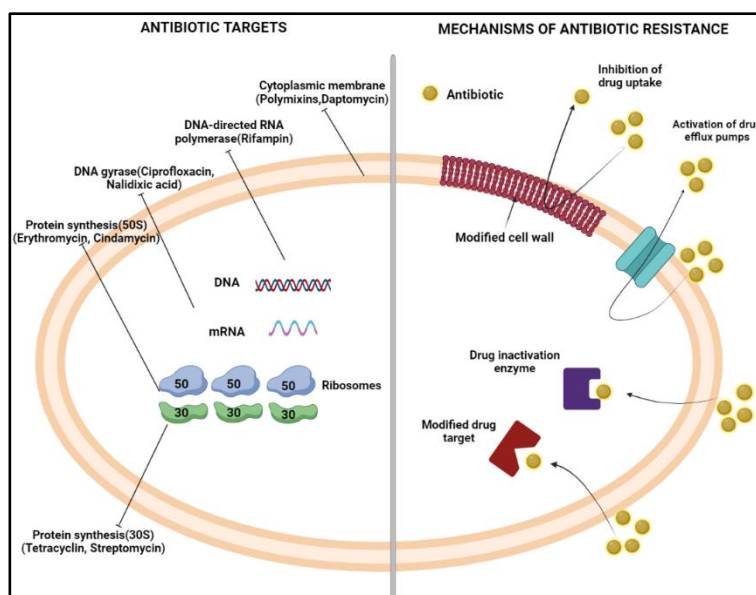


Figure 2.3: Regulation of virulence determinants in *S.aureus*

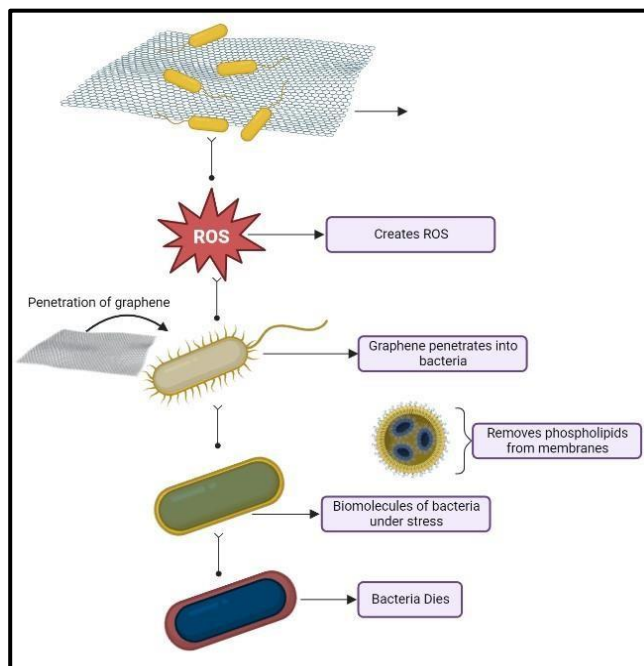


Figure 2.4: Mechanism of graphene's lethal activity against prokaryotes

(Created by using Biorender)

Figure Description: When the surface of graphene comes in contact with bacteria, it stimulates the bacteria to release a few Reactive Oxygen Species (ROS) like H_2O_2 and O_2^- . Then this graphene breaches into the prokaryotic system and disintegrates the structural integrity of the membrane by removing the phospholipid. Which then by the cascade of process kills the bacteria.

2.4.2 Graphene's action against phytopathogens

Recently, a remarkable use of graphene in biological research has been proposed, to manage plant infections. It is believed that graphene, namely GO, has unique optical, electrical, mechanical, and thermal properties that contribute to its antibacterial activity. These properties include ease of surface modification, high mechanical strength, great water dispersibility, and photoluminescence (V. Singh *et al.*, 2011). This broadened the application of graphene, which can be used for the management of agricultural diseases. Around the globe, crop diseases brought on by a diverse range of phytopathogenic fungi and bacteria may lower a cultivar's yield by 20–40%. Multidrug-resistant infections have been brought about by the formation of strong, unbreakable resistance in microorganisms as a consequence of continuous and extensive exposure to numerous bactericides and antibiotics (Rezzonico *et al.*, 2009). The question of whether graphene oxide has a wide range of antibacterial activity against plant diseases is one worry with employing it as an antimicrobial agent.

To understand the mechanism of interaction between GO, a detailed investigation into the antibacterial activity of graphene oxide (GO) against two bacterial pathogens (*P. syringae* and *X. campestris pv. undulosa*) and two fungal diseases (*F. graminearum* and *F. oxysporum*) was conducted. The results showed that GO had a significant effect on the growth of all four pathogens (at 500 mg mL^{-1} , GO killed roughly 90% of the bacteria and reduced 80% of macroconidia germination along with partial cell swelling and lysis). This study postulated a cooperative mechanism in which a variety of aggregated graphene oxide sheets interact with the vital activity of both

bacterial and fungal spores to damage local cell membranes, reduce bacterial membrane potential, and cause electrolyte leakage from fungal spores (J. Chen *et al.*, 2014). Cell lysis may be one of GO's primary cytotoxic effects on phytopathogens. This is likely how GO interacts with the pathogens by physically encircling, damaging the pathogens locally, and eventually causing cell lysis.

2.4.2.1 Mechanism of interaction (between fungus and rGO)

Reduced graphene oxide (rGO) and fungus interact in a complicated manner via several processes. Physical and chemical interactions between fungi and rGO may occur, and these interactions can be impacted by a variety of variables, including the size and form of the rGO, the kind of fungus involved, and the surrounding environment. Adsorption is one of the main ways that fungus and rGO interact with one another. Many oxygen-containing functional groups, including carboxylic and hydroxyl groups, are present on the surface of rGO and may interact with the fungal cell wall (Yu *et al.*, 2015). The growth, development, and metabolism of the fungus may vary as a result of the adsorption of rGO onto the fungal cell wall due to changes in the shape, structure, and permeability of the cell wall. Transferring electrons is another method by which fungus and rGO interact. rGO can move electrons to or from the fungus cells thanks to its strong electron conductivity. The redox balance of the fungal cells and subsequent effects on metabolism, growth, and survival may result from this electron transfer. Reactive oxygen species may also be produced as a result of the interaction between fungus and rGO (ROS). ROS are very reactive chemicals that may harm DNA, proteins, and the fungal cell membrane. ROS may be produced as a result of rGO adhering to the fungal cell wall, which can cause oxidative stress and cell death (Reshma *et al.*, 2017).

Overall, adsorption, electron transfer, and ROS formation are some of the mechanisms that may be involved in the complicated mechanism of interaction between fungus and rGO. Depending on the particular fungus species and the characteristics of the rGO used the exact nature of the interaction may change (Radhi *et al.*, 2021).

2.4.3 Interaction of graphene with plants

Most studies have focused on how toxicity might accumulate in plant roots, but little is known about how it can spread to vascular tissues or the risks associated with modified graphene materials. In this work, we used a ^{13}C -stable isotope skeleton-labeling technique to investigate the translocation of two graphene materials in pea plants (*Pisum sativum* L.) and their effects on leaf photosynthesis (L. Chen *et al.*, 2019). Chemical reduction of graphene oxide (GO) produced reduced graphene oxide (rGO), which was carried from the roots to the leaves and directly hampered the function of photosystem II (PS II) by damaging the oxygen-evolving complex on the donor side, a condition known as oxidative stress. These results emphasize the importance of chemical reduction in the translocation of graphene compounds in plants as well as the risks associated with such materials interfering with critical metabolic processes in the carbon cycle. On the other hand, non-reduced GO was restricted to plant roots and did not affect photosynthesis. This study underlines the importance of the chemical structure of rGO nanosheets and its safe interaction with higher plant cells. The potential harm to plant photosynthesis and carbon fixation should be appropriately considered while producing and using graphene products (J. Singh and Sengar, 2020).

2.4.3.1 Graphene as a germination promoter

Plant seed germination requires the right temperature, together with enough moisture and aeration. At the commencement of germination, the hydrotropic components of the seed begin to absorb water. They then continue to do so as the embryo grows. As a soil additive for moisture retention, GO was tested on the germination of spinach and chive. The popular infant meal spinach has a high nutritional content, and the ubiquitous spice chive helps with digestion and absorption. The hydrophilic character of GO and its ability to carry water aided in hastening plant germination by a little dosage. The reason for this was that the hydrophobic sp^2 domains of GO were used to transport water molecules to the seeds from the oxygen-containing functional groups of GO (Y. He *et al.*, 2017). The combination of the sp^2 and

sp³ structures of GO gives it special abilities to function as a potent water transporter in soil. Furthermore, GO is attracted to soil particles and repels plant roots electrostatically, preventing them from building up in plants. The plants produced in this way pose no harmful threat. Given the number of oxygen-containing functional groups and the size of its particular surface, GO has great potential as a nano-fertilizer to promote plant growth (Zhao *et al.*, 2011).

2.5 *IN-SILICO* ANALYSIS OF REDUCED GRAPHENE OXIDE

With the advent of computer simulations and modeling tools for materials science research, *in-silico* investigation of reduced graphene oxide (rGO) has only recently become a reality. Graphene research, on the other hand, has a long history that dates back to the 1940s. P.R. Wallace predicted the presence of a two-dimensional structure composed of carbon atoms organized in a hexagonal lattice, which led to the discovery of graphene in 1947 (Tiwari *et al.*, 2018). Yet it was not until the 2000s that graphene was isolated and scientifically defined, sparking a boom in research interest in this substance. A derivative of graphene called reduced graphene oxide (rGO) is created by reducing graphene oxide (GO), which is created by oxidizing graphite (Ray, 2015). The majority of the oxygenated functional groups are removed during the reduction of GO to rGO, which also improves the material's mechanical and electrical conductivity by restoring some of the sp² carbon-carbon bonds.

Using simulations and computational techniques to analyze the characteristics and behavior of the material is referred to as an *in-silico* study of rGO. Studying the electrical structure, mechanical characteristics, and interactions with other materials are a few examples of this (Piras *et al.*, 2021). Understanding the characteristics of rGO and its prospective uses in a variety of industries, including electronics, energy storage, and sensors, requires the use of *in-silico* analysis. Recent years have seen a significant increase in the use of *in-silico* analysis for rGO research, with many studies concentrating on streamlining the reduction process, enhancing the characteristics of rGO, and creating new uses for this material. *In-silico* research is anticipated to

become an increasingly crucial tool for the study of rGO and other graphene derivatives as computational power and simulation methods develop (Wang *et al.*, 2017).

Reduced graphene oxide (rGO) *in-silico* analysis has several potential uses in numerous industries. Here are a few illustrations:

2.5.1 Electronic devices

Based on rGO, electronic devices such as field-effect transistors, sensors, and energy storage devices may be designed and optimized via *in-silico* analysis (Ahmed and Yi, 2017). This includes researching rGO's electrical structure, transport characteristics, interactions with other materials, and more.

2.5.2 Energy storage

rGO is a potential material for energy storage applications because of its large surface area, electrical conductivity, and mechanical robustness. The performance of rGO-based supercapacitors and batteries may be studied, and the material parameters can be optimized for improved performance, using *in-silico* research (Liao *et al.*, 2021).

2.5.3 Catalysis

It has been shown that rGO possesses catalytic activity for several chemical processes, including oxygen reduction and hydrogen evolution. The catalytic capabilities of rGO may be investigated, and its composition and structure can be tailored for certain reactions using *in-silico* studies.

2.5.4 Water treatment

rGO is successful in purifying water of impurities such as organic pollutants and heavy metals. The interactions between rGO and water molecules may be studied using *in-silico* research, and the material characteristics can be improved for better water treatment performance (Pérez-Ramírez *et al.*, 2016).

2.5.5 Biomedical applications

rGO may be used in tissue engineering, bioimaging, and medication delivery. As well as optimizing the material's characteristics for particular biomedical applications, the *in-silico* analysis may be utilized to examine the interactions between rGO and biological molecules.

Overall, rGO *in-silico* analysis has the potential to speed up the creation of novel applications for this substance and to direct experimental research for a deeper comprehension of its characteristics and behavior.

A computer technique called "*in-silico* docking" is used to forecast how tiny compounds (or "ligands") will interact with proteins or macromolecular complexes (receptors). Predicting the binding affinity and position of the ligand inside the receptor's active region requires modeling the interaction between the ligand and receptor.

3. AIM AND SCOPE

- To analyze the physical characteristics of reduced graphene oxide synthesized.
- To recognize which surface property of graphene family nanomaterials elicits the toxicity phenomena over lower organisms.
- To formulate the accurate concentration of reduced graphene oxide for substituting it as the chemical fertilizer and as an antibiotic with minimal side effects.
- To develop a toxicity pathway of the reduced graphene oxide over the *Staphylococcus aureus*, *Escherichia coli* and *Proteus* bacteria and various fungal phytopathogens namely, *Colletotrichum gloeosporioides*, *Fusarium oxysporum* f.sp. *cubense*, *Nigrospora oryzae*, *Athelia rolfsii*, *Pyricularia oryzae* and *Curvularia lunata*.
- To compare the concentrations of Reduced graphene oxide(rGO) at which the growth of bacteria and phytopathogens are inhibited.
- To dock rGO nanosheets with various prokaryotic membranous proteins and specific cancerous proteins of higher mammals and support the toxicity pathway developed.
- To perform in-silico analysis of various anticancer and over the counter drug to check the ability of rGO nanosheets to act as drug carrier for targeted drug delivery.

4. MATERIALS AND METHODS

4.1 SYNTHESIS OF GRAPHENE NANOSHEETS AS rGO

Hummer's modified method was used to create rGO from graphite flakes (Deemer *et al.*, 2017). The solution of concentrated sulphuric acid and phosphoric acid, graphite flakes, and the necessary quantity of potassium permanganate (KMnO_4) along with sodium nitrate (NaNO_3) as an oxidizing agent was used in the improved technique (9:1). When the mixture had been properly diluted with ice-cold distilled water and was kept at a temperature below 35°C for 24 hours with steady stirring, 30% hydrogen peroxide was added. The brown GO was then slowly reduced to rGO by refluxing it at 100°C with hydrazine hydrate. The product was then centrifuged, cleaned at least four times with distilled water to remove any remaining contaminants, and dried under a vacuum for a whole night at 80°C . The modified procedure employed by Hummers included KMnO_4 as well as an equal quantity of (Sahudin *et al.*, 2021).

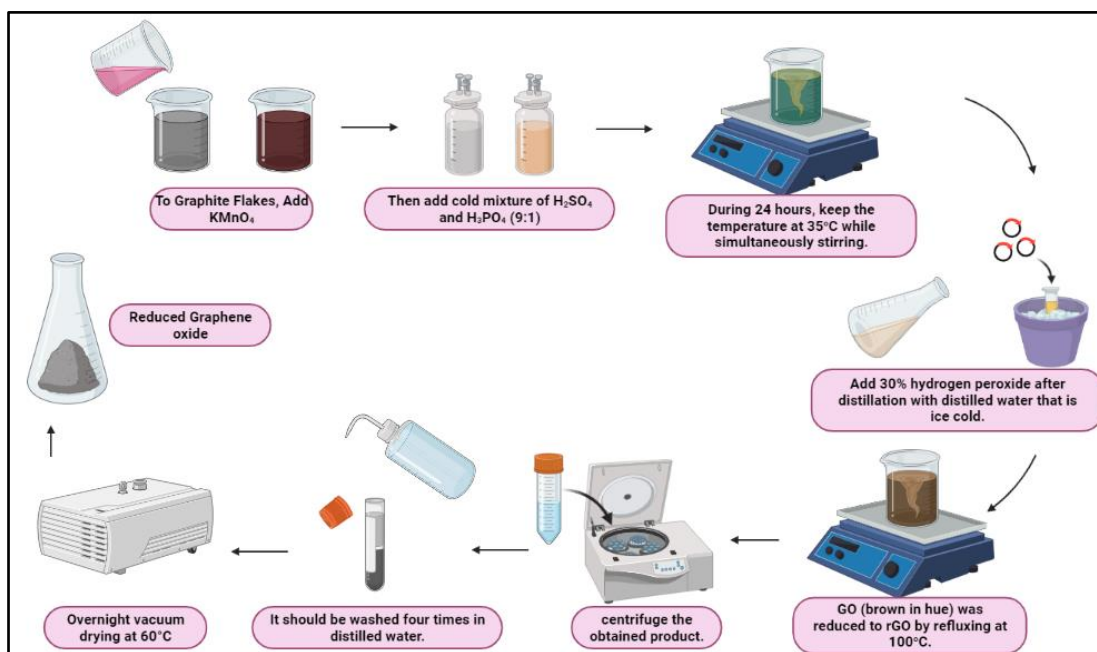


Figure 4.1: Schematic flowchart of synthesis of rGO nanosheets by Modified Hummer's method (Created using Biorender)

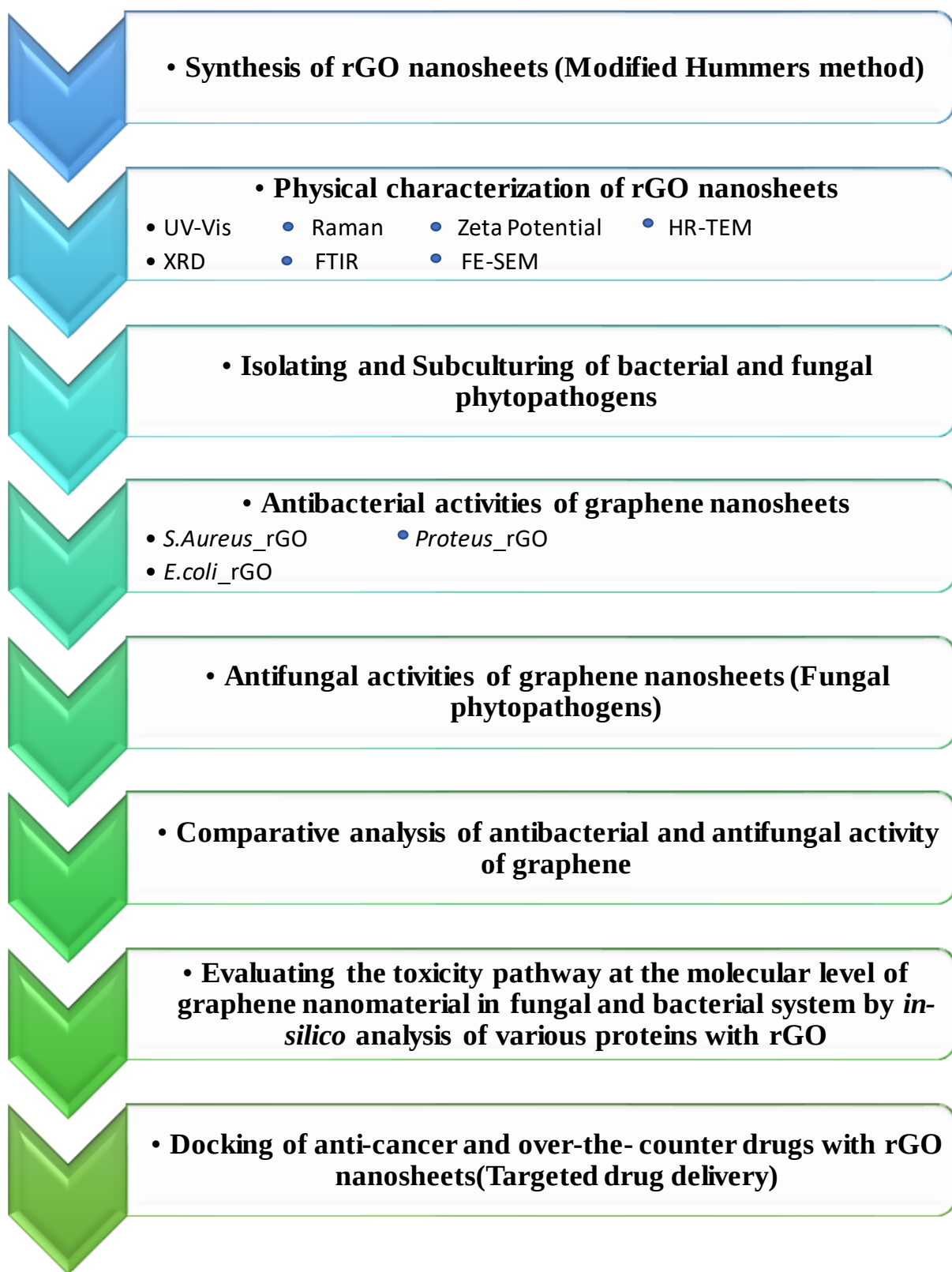


Figure 4.2: Flowchart of Methodology

4.2 PHYSICAL CHARACTERIZATION OF GRAPHENE NANOSHEETS

4.2.1 UV-Visible Spectrometry

An analytical technique suitable for a large class of organic chemicals as well as certain inorganic species, UV-vis spectroscopy is affordable, straight forward, adaptable, and non-destructive (Pons *et al.*, 2004). The absorbance or transmittance of light flowing through a material as a function of wavelength is measured using UV-vis spectrophotometers. UV-vis detectors are integrated into high-performance liquid chromatography and ultra-high-performance liquid chromatography to detect and measure the concentration of chemicals in liquid streams (Rocha *et al.*, 2018). All species identification is facilitated by combining these methods with mass spectrometry. A variation that has improved scattering capabilities for measuring the characteristics of solids and powders is UV-vis diffuse reflectance spectroscopy. The reduced graphene oxide (rGO) that we created using a modified Hummers technique was quantified for this experiment using a Shimadzu UV-1800 purchased from Japan (Piovesan *et al.*, 2018).

Graphene nanosheets may be examined using UV-Visible (UV-Vis) spectroscopy. For this, the following methods may be applied:

4.2.1.1 Sample preparation

An appropriate technique, such as the chemical reduction of graphene oxide, may be used to create graphene nanosheets. To create a stable suspension, the manufactured graphene nanosheets should be mixed with a suitable solvent, such as water or ethanol.

4.2.1.2 Instrument setup

Setting up the UV-Vis spectrophotometer involves modifying variables including baseline correction, slit width, and wavelength range.

4.2.1.3 Measurement

The produced suspension of graphene nanosheets is put into the sample holder of a UV-Vis spectrophotometer, and an appropriate wavelength range is selected for recording the absorbance spectra. The graphene nanosheets' electrical structure and optical characteristics are revealed through the absorbance spectrum.

4.2.1.4 Analysis

The resultant absorbance spectrum may be examined to ascertain the bandgap, number of layers, and degree of oxidation of the graphene nanosheets, among other optical characteristics. The analysis may be carried out using software techniques like peak deconvolution or Gaussian fitting, or it can be done by comparing the resulting spectrum with reference spectra.

It is vital to remember that the stability and quality of the suspension of graphene nanosheets as well as the settings for the instrument may have a big impact on how accurate and repeatable the UV-Vis readings are. To get accurate findings, careful sample preparation and calibration of the equipment are required (Zare *et al.*, 2022).

4.2.2 X-Ray Diffraction

The method of X-ray diffraction (XRD) is often used to describe the composition and characteristics of nanoparticles (da Silva *et al.*, 2011). While the XRD process for nanoparticles is similar to that for bulk materials because, to the nanoparticles' tiny size, several unique considerations must be made. The following are the general stages involved in the XRD analysis of nanoparticles:

4.2.2.1 Sample preparation

The first step is to create a sample of nanoparticles that is evenly distributed. This may be accomplished by utilizing sonication or other techniques to disperse the

nanoparticles in a suitable solvent, such as water or ethanol. Any impurities or pollutants that might taint the XRD findings should not be present in the sample.

4.2.2.2 *Instrumentation*

A high-intensity X-ray beam with a tiny spot size should be able to be produced by the XRD apparatus used for nanoparticle investigation. For the X-rays to be diffracted from the nanoparticles and not the substrate or any other contaminants in the sample, this is important.

4.2.2.3 *X-ray diffraction*

A detector then picks up the dispersed X-rays after the sample has been subjected to a high-intensity X-ray beam. The nanoparticles' crystal structure may be recognized by the X-ray diffraction pattern that was produced.

4.2.2.4 *Data Analysis*

The crystal structure of the nanoparticles is then determined by analyzing the XRD pattern. This entails comparing the pattern's peaks to those in a database of crystal structures that are already known to exist. With the use of several analytical techniques, such as the Scherrer equation, the size, and form of the nanoparticles may also be ascertained from the XRD pattern.

4.2.2.5 *Interpretation of results*

The XRD data may provide details on the nanoparticles' size, shape, and orientation as well as their crystal structure. The production of nanoparticles may be made more efficient using this knowledge, and their characteristics and behavior can be better understood (Manik *et al.*, 2020).

To establish the crystal structure, size, and form of the nanoparticles, an XRD examination of nanoparticles entails the preparation of a well-dispersed sample,

exposure to a high-intensity X-ray beam, analysis of the resultant diffraction pattern, and interpretation of the findings.

4.2.3 Raman Spectrometry

An effective technique for characterizing nanoparticles is Raman spectroscopy. The technique of Raman spectroscopy for powdered nanoparticles is similar to that used for bulk materials, but owing to the tiny size of the particles, certain special considerations need to be made (Li Bassi *et al.*, 2005). The following are the general stages involved in the examination of powdered nanoparticles using Raman spectrometry:

4.2.3.1 Sample preparation

The first step is to make a sample of nanoparticles that is powdered. This may be accomplished by using a ball mill or a mortar and pestle to ground the nanoparticles to a tiny powder. Any impurities or pollutants that might taint the Raman findings should not be present in the sample.

4.2.3.2 Instrumentation

A high-intensity laser beam with a tiny spot size should be able to be produced by the Raman spectrometer utilized for nanoparticle investigation. Making sure the laser only interacts with the nanoparticles and not the substrate or any other contaminants in the sample, is essential. To achieve a smooth surface, the powdered sample is typically put onto a flat sample holder and leveled off (Slavov *et al.*, 2010).

4.2.3.3 Laser excitation

After then, the sample is subjected to a powerful laser beam, usually in the visible or near-infrared spectrum. The nanoparticles shake as a result of the laser activation, creating a Raman scattering signal.

4.2.3.4 *Raman Spectroscopy*

A spectrometer is used to gather and examine the dispersed light. The nanoparticles' vibrational modes are characterized by the Raman spectrum that was acquired.

4.2.3.5 *Data Analysis*

The content and structure of the nanoparticles are then determined by examining the Raman spectra. To do this, the peaks in the spectrum are compared to those in a database of well-known Raman spectra.

4.2.3.6 *Interpretation of results*

The Raman results may provide details about the nanoparticles' chemical and physical makeup, size, shape, and content. The production of nanoparticles may be made more efficient using this knowledge, and their characteristics and behavior can be better understood.

In conclusion, the Raman spectrometry analysis of powdered nanoparticles entails preparation of a fine powder sample, exposure to a high-intensity laser beam, analysis of the resulting Raman spectrum, and interpretation of the results to ascertain the composition, size, and shape of the nanoparticles, as well as their chemical and physical properties (Li *et al.*, 2018).

4.2.4 Zeta potential

For the determination of the zeta potential of suspended nanoparticles, the Horiba Nanopartica Zetasizer is a commonly used tool. For powdered nanoparticles, the following stages are used in the process for measuring zeta potential using the Horiba Nanopartica Zetasizer machine:

4.2.4.1 *Sample preparation*

The powdered nanoparticles must first be dispersed in a suitable solvent to create a stable suspension. Sonication or mechanical stirring may be used to accomplish this. Based on the characteristics of the nanoparticles and their planned usage for them, the solvent should be selected.

4.2.4.2 *Instrumentation*

A measuring cell that is intended for zeta potential measurements is part of the Horiba Nanopartica Zetasizer machine. The device measures the zeta potential and size of the suspended nanoparticles using dynamic light scattering (DLS) (Jain *et al.*, 2020).

4.2.4.3 *Setting up the machine*

The measuring cell is cleaned before use, and the device is calibrated. After loading the sample into the measuring cell, the apparatus is prepared for zeta potential measurement.

4.2.4.4 *Measurement of zeta potential*

The device exposes the sample to an electric field and gauges how the particles move as a consequence. Usually, it takes a few minutes to finish the measurement. The Smoluchowski equation or other appropriate equations are then used to compute the zeta potential using the observed electrophoretic mobility.

4.2.4.5 *Interpretation of results*

Information on the stability and colloidal behavior of the nanoparticles in the suspension may be gleaned from the zeta potential measurements. When the zeta potential is high, the nanoparticles are evenly distributed and stable, but when it is low, the nanoparticles are more likely to aggregate or settle. The outcomes of the zeta

potential analysis may also be utilized to improve nanoparticle manufacturing and formulation for a variety of applications.

In conclusion, the Horiba Nanopartica Zetasizer machine can measure the zeta potential of powdered nanoparticles by dispersing them in a suitable solvent, loading the sample into the measurement cell, applying an electric field, measuring the zeta potential using dynamic light scattering, and interpreting the results to determine the stability and colloidal behavior of the nanoparticles in suspension (Brandhonneur *et al.*, 2018).

4.2.5 FTIR

The measurement of a sample's infrared radiation transmission or absorption using FTIR spectroscopy is a method often used to characterize nanoparticles in terms of their chemical makeup and functional groups (Petit and Puskar, 2018). An expert tool, such as an FTIR spectrophotometer, is needed to conduct FTIR spectroscopy on nanoparticle samples.

The procedures listed below may be used to conduct FTIR spectroscopy on samples of powdered nanoparticles using an FTIR spectrophotometer (S. Yang *et al.*, 2021):

4.2.5.1 *Prepare the sample*

The sample of powdered nanoparticles has to be combined with an appropriate infrared-transparent matrix, such KBr or Nujol. A hydraulic press is then used to shape the mixture into a thin pellet.

4.2.5.2 *Place the pellet in the sample holder*

The Shimadzu UV-1800 spectrophotometer's sample container is filled with the pellet, and the device is then mounted with the sample holder inside.

4.2.5.3 *Collect the spectrum*

The FTIR spectrophotometer is set up to scan the sample across several wavelengths to obtain the spectrum. In transmission mode, when infrared light passes through the sample and is detected on the opposite side, the spectrum is normally gathered.

4.2.5.4 *Analyze the spectrum*

The functional groups present in the sample and the nanoparticles' chemical makeup are both determined by analyzing the acquired FTIR spectrum.

4.2.6 **HR-TEM**

A method for seeing the atomic-scale structure of materials, including nanoparticles, is High-Resolution Transmission Electron Microscopy (HRTEM) (Hrnjic *et al.*, 2021). The approach that may be utilized to conduct HRTEM analysis on samples of powdered nanoparticles is as follows:

4.2.6.1 *Sample preparation*

A drop of the solution is applied to a thin copper grid that has been coated with carbon after the powdered rGO nanoparticle sample has been dissolved in a solvent such as ethanol or isopropanol. On the grid, a small layer of nanoparticles is left behind when the solvent evaporates.

4.2.6.2 *Instrument setup*

The HR-TEM apparatus, which is normally a highly specialized and sensitive microscope, is then filled with the carbon-coated copper grid. Next, by altering the magnification, beam intensity, and other settings, the equipment is prepared for imaging.

4.2.6.3 *Imaging*

A beam of electrons is then used to photograph the nanoparticle sample by passing through it and landing on a detector on the opposite side. The atoms in the sample scatter the electrons as they go through it, giving rise to a picture of the nanoparticles' atomic structure.

4.2.6.4 *Image analysis*

Using specialist software, it is possible to identify the size, shape, and crystal structure of the nanoparticles from the HR-TEM pictures.

It is crucial to remember that HR-TEM is a highly specialized method that calls for specialist tools and knowledge to execute successfully. To guarantee that high-quality pictures are acquired, it is crucial to carefully manage the sample preparation, instrument setup, and imaging settings.

4.2.7 FESEM

With the help of the field emission scanning electron microscope (FE-SEM), it is possible to get high-resolution images of the surfaces of many materials, including nanoparticles (Dalgleish *et al.*, 2004). The approach listed below may be used to conduct FESEM analysis on samples of powdered nanoparticles:

4.2.7.1 *Sample preparation*

A drop of the suspension is applied to an appropriate substrate, such as a silicon wafer, after the powdered rGO nanoparticle sample has been dissolved in a solvent like ethanol or isopropanol. A tiny layer of nanoparticles is left on the substrate after the solvent has been allowed to evaporate.

4.2.7.2 *Instrument setup*

The rGO nanoparticle sample substrate is then put into the FE-SEM device. Next, by altering the magnification, beam energy, and other settings, the equipment is prepared for imaging.

4.2.7.3 *Imaging*

A beam of electrons is then focused onto the sample's surface to scan the rGO nanoparticle sample. A detector in the FE-SEM equipment picks up secondary electrons that are released as a result of the electrons' interactions with the sample's atoms. The generated photos provide high-resolution details on the shape and surface structure of the nanoparticles.

4.2.7.4 *Image analysis*

Using specialist software, it is possible to examine the FESEM pictures and identify the size, shape, and surface characteristics of the nanoparticles.

4.3 ANTI-BACTERIAL ASSAY

Bacterial strain, *S. aureus*, *Proteus*, and *E. coli* which was isolated and subcultured was exploited for implementing the antibacterial activity. The confirmation of these bacteria was done by microscopic analysis. After isolation, they were subcultured and stored in Nutrient agar, MacConkey agar, and Muller Hinton Agar respectively.

The activity was checked by preparing three different agars separately and sterilizing them. Thereafter the prepared media was poured onto the Petri plates, the bacteria culture was swabbed in the agar-solidified Petri plates. Then four wells were made using sterilized cork corer. The wells were filled in an order of Penicillin (which acted as control), 100µg/ml, 200µg/ml, and 400µg/ml of powdered rGO nanosheets which were dissolved in distilled water by sonication. All the plates were incubated at

37°C for 24 hours. After which zone of inhibition (ZOI) was visible as depicted in the results.

4.4 ISOLATING AND SUBCULTURING THE PHYTOPATHOGENS

4.4.1 Identifying and isolating the fungal phytopathogens

Twenty grams of soil sample was collected from the Banana field in Embalam, Puducherry was collected at a depth of 15 cm. It was then shade dried. One gram from the twenty grams of soil collected was weighed using an electronic weighing machine and was transferred to 9 ml of distilled water which is marked as 10^{-1} , this marks the beginning of the serial dilution process (Cao *et al.*, 2004; Nel *et al.*, 2006). The serial dilution was performed where 1ml from a 10^{-1} test tube was transferred using a micropipette to a 10^{-2} test tube and the contents were mixed uniformly using a vortex. Then the process was further continued in the same way until it reached 10^{-10} test tubes. 20 Now, 1ml using micropipettes from 10^{-3} , 10^{-4} , and 10^{-5} was poured aseptically onto sterile Petri plates (90mm). Then 10 mL of Potato Dextrose Agar medium was poured into those Petri plates and after solidification, the plates were kept was kept for 5 days during the incubation period (Chand Kumhar, 2014).

The symptomatic banana leaf and trunk samples were collected from the field at Embalam, Puducherry. Then the sample isolation technique was adopted where the infected parts were cut using a sterile blade and washed with running tap water and blot dried. Then they were surface sterilized with 0.1% mercuric chloride for 1 min and again soaked immediately in sterile distilled water for 30 secs. The surface-sterilized infected parts of the root were placed near the edge of the Petri plates containing sterilized and solidified Potato Dextrose Agar (PDA) medium under a laminar flow hood. Then it was placed on the solidified potato dextrose agar medium and was left for incubation (García-Bastidas *et al.*, 2014). A similar process was carried out for other fungal phytopathogens.

4.4.2 Subculturing the identified fungal phytopathogens

The fungal phytopathogens that were isolated from the incubated soil sample collection plate which was used for confirming the presence of pathogen were transferred to the Petri plate with solidified potato dextrose agar using a cork-borer under the laminar flow hood. Then this was kept for incubation for the proper growth of the pathogen that can be used for further studies (Yassin *et al.*, 2021).

4.5 ANTIFUNGAL ASSAY

rGO nanosheets were dispersed in 20 ml PDA agar at 50°C and poured into sterilized Petri dishes (90 mm in diameter). After solidification, agar disks (5 mm diameter) covered by fungal growth were aseptically removed from a young (5-day-old) PDA fungal culture. The disks were aseptically placed at the center of the PDA agar plates containing rGO nanosheets (from control with 0µg/20ml to 600µg/20ml, depending upon the standardized concentration for each phytopathogen). All Petri plates were incubated at 25°C for 7 days. Experiments were performed with four replicates. The average fungal colony diameters were determined. Growth inhibition of phytopathogens was proportional to the concentrations of the rGO nanosheets in PDA.

4.6 IN-SILICO ANALYSIS

Reduced graphene oxide nanosheets were created using ChemDraw software, where the nanoparticle can be used as a ligand while docking with specific proteins and protein while docking it with specific anti-cancer and over-the-counter drugs for performing targeted drug delivery in higher organisms.

The stages followed for the docking process:

4.6.1 Preparation of the ligand and receptor structures

In molecular docking studies, the creation of the ligand and receptor structures is crucial. The molecule that is docking into the receptor, which is often a protein or nucleic acid molecule, is referred to as the ligand. Predicting the binding manner and affinities of the ligand to the receptor is the aim of molecular docking. Getting the molecules' three-dimensional coordinates is the initial step in creating the ligand and receptor structures. Experimental techniques like X-ray crystallography, NMR spectroscopy, or electron microscopy may be used to do this. Other methods for obtaining the structures include computational molecular modeling methods like ChemDraw software (Guedes *et al.*, 2014).

4.6.2 Grid generation

Grid generation, which entails building a three-dimensional grid of dots to depict the receptor molecule's possible binding site, is an essential stage in the molecular docking process. The grid determines the search space where the ligand will dock and aids in determining the best mode of binding and receptor affinity for the ligand. The ligand molecule is docked into the grid's predetermined search space once the grid has been constructed. The grid enables the effective computation of the receptor-ligand interaction energies, which are used to foretell the best binding mode and affinity of the ligand to the receptor (Elokely and Doerksen, 2013).

4.6.3 Ligand docking

A crucial phase in the molecular docking process is ligand docking, in which a ligand molecule is positioned in a receptor molecule's binding site and the interaction between the two molecules is anticipated. The purpose of ligand docking is to discover prospective therapeutic candidates for further experimental validation and to predict the binding mechanism and affinity of the ligand to the receptor. The ability to anticipate the binding mechanism and affinity of ligand molecules to receptor molecules makes ligand docking a potent computational tool that may be utilized for drug development as well as other molecular biology and biochemistry applications.

4.6.4 Scoring

In molecular docking, scoring entails determining the anticipated binding affinity of a ligand to a receptor. The objective of scoring is to rank the ligands according to their expected binding affinities and to locate the ligand-receptor complex with the highest advantageous interaction energy (Krovat *et al.*, 2005).

4.6.5 Analysis

To understand the interactions between ligands and receptors and to find prospective therapeutic candidates, analysis is a crucial stage in the molecular docking process. It is essential to analyze the docking data to comprehend how ligands and receptors interact as well as to find promising medication candidates. This information can be used to inform the design and optimization of ligand molecules for drug discovery and other uses in molecular biology and biochemistry. It can also give insight into the binding mode, binding affinity, selectivity, and other important characteristics of the ligand-receptor complex (Morrone Xavier *et al.*, 2016).

In-silico docking is utilized in drug discovery and design because it enables scientists to forecast how tiny compounds will attach to certain proteins and to refine the structures of those molecules to increase their binding affinity and selectivity. Reduced graphene oxide (rGO) and proteins may interact, and one computer technique to anticipate this interaction is called *in-silico* docking. The procedure entails modeling the interaction between the protein and the rGO surface and making predictions about the protein's affinity for binding and its orientation within the surface (Rao and Srinivas, 2011). To forecast the interactions between them, reduced graphene oxide (rGO) may be docked with both ligands and proteins individually.

When an rGO and a ligand are docked, the interaction between the two is simulated, and the binding affinity and orientation of the ligand inside the rGO surface are predicted. Since it enables scientists to foresee the ideal ligands for functionalizing the surface of rGO and improves their targeting and therapeutic effectiveness, this kind

of docking is often utilized in drug discovery and design. The method of docking rGO with a protein, on the other hand, entails modeling the interaction between the protein and the rGO surface and predicting the binding affinity and orientation of the protein inside the rGO surface. Due to its ability to aid in the creation of innovative graphene-based materials for biomedical applications including biosensors, drug transport, and tissue engineering, this kind of docking is often utilized in the fields of nanobiotechnology and drug discovery. In general, *in-silico* docking of rGO with either ligands or proteins may provide insightful information on the nature of interactions between rGO and biomolecules and aid in the development of novel materials and applications.

5. RESULTS

5.1 SYNTHESIS OF REDUCED GRAPHENE OXIDE

Using the modified hummers method reduced graphene oxide nanosheets were successfully synthesized in powdered form.

5.2 PHYSICAL CHARACTERIZATION OF GRAPHENE NANOSHEETS

5.2.1 UV-Visible Spectrometer (Shimadzu UV-1800, Japan)

For the confirmation of the synthesized nanoparticle, the rGO powder was subjected to UV-visible spectroscopy. There was a peak identified at 300 to 400 nm which is depicted in *Figure 5.1*. This result confirms that the particle synthesized is reduced to graphene oxide nanosheets.

5.2.2 X-Ray Diffraction (Rigaku MiniFlex--II Desktop X-ray diffractometer)

XRD has been employed for studying the diffraction angle at which characteristic crystalline peaks are formed for MRGO nanosheets. *Figure 5.2* describes the XRD patterns of MRGO. During the process of XRD, exfoliation had taken place from graphite to GO, and then further removal of oxygen groups from Intercalating layers of GO occurred. This resulted in the formation of (002) crystalline peaks at 24.3 Å of X-ray imparted. The additional appearance of a small diffraction peak of (111) at 42.8 Å reveals the short-range order in stacked graphene layers.

5.2.3 Raman Spectroscopy (Renishaw inVia Raman Microscope)

This particular physical characterization has been carried out to study the crystallinity, disorder, and defect in rGO nanosheets. The results of this analysis are depicted in *Figure 5.3*. G peak at 1600 cm^{-1} , this is attributed due to the re-graphitization

of the carbon network resulting in the creation of a large number of sp^2 carbon species in the graphene network and D peak is at 1400 cm^{-1} . Using the relative intensity ratio, that is I_D/I_G , the strength of defects present in the crystal lattice of a material can be evaluated. The I_D/I_G for MRGO is 1.375 ($I_D/I_G = 110000/8000 = 1.375$). This shows that the concentration of defects is higher than normal, which can result in the formation of many internal benzene-derived sp^2 domains in a distinctive graphene structure.

5.2.4 Zeta potential (Horiba Nanopartica Zetasizer)

The zeta potential of reduced graphene oxide nanosheets has been studied in an aqueous medium where an rGO powdered sample was dissolved with distilled water by sonicating it, to inspect the colloidal stability of nanomaterials in the dispersion state. The zeta potential mean value is -47 mV as shown in *Figure 5.4*, which is greatly above the acceptable limit of colloidal stability in the dispersion state.

5.2.5 FTIR (Agilent Cary630 FTIR)

To understand the major functional groups, present in the rGO nanosheet synthesized, FTIR studies have been carried out, the result is depicted in *Figure 5.5*. The broad and intense peak of wavenumbers at $\sim 3000\text{ cm}^{-1}$ arising from ($-\text{OH}$) stretching vibration, a peak at $\sim 1715\text{ cm}^{-1}$ due to ($-\text{C}=\text{O}$) stretching vibrations of carboxylic groups and sharp $\text{C}=\text{C}$ band within the range of 1500 cm^{-1} .

5.2.6 HR-TEM ((S)TEM Microscope, Talos F200S G2 TEM)

The morphology of rGO nanosheets synthesized is characterized by HR-TEM analysis. The results depicted in *Figure 5.6* indicates the presence of wrinkles and folded regions on the surface of rGO. The images were collected at 10nm (*Figure 5.6(a)*), 50nm (*Figure 5.6(b)*), and 100nm (*Figure 5.6(c)*) to find out the intensity of folds present on the surface of rGO.

5.2.7 FE-SEM (Carl Zeiss NTS GMBH, SUPRA 55)

FE-SEM images (Figure: 5.7) of rGO synthesized by the modified hummers method depict the sharp edges, which proves its toxicity over lower organisms such as bacteria and fungal phytopathogens, as this saw-like surface can cut open the peptidoglycan layer of bacteria and a chitinous layer of fungus easily, which in turn will stimulate reactive oxygen species and result in the death of the organisms.

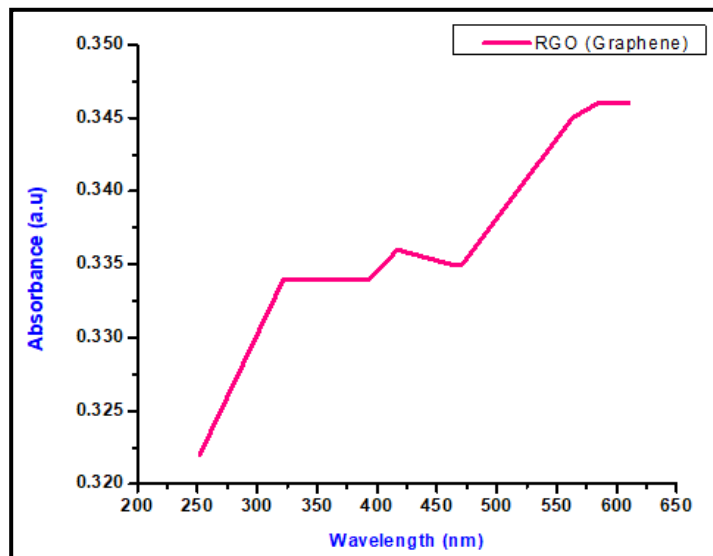


Figure 5.1: UV- Visible spectroscopy results of rGO

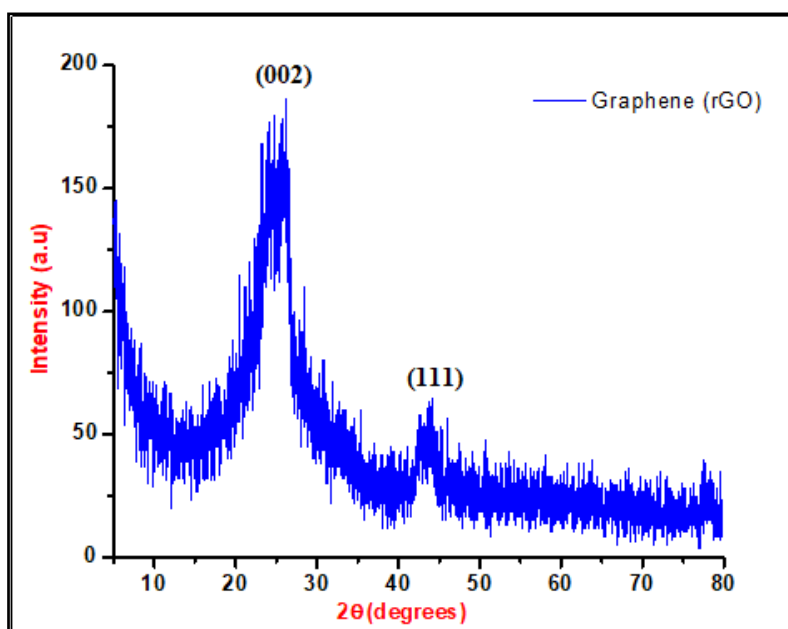


Figure 5.2: XRD peaks of rGO

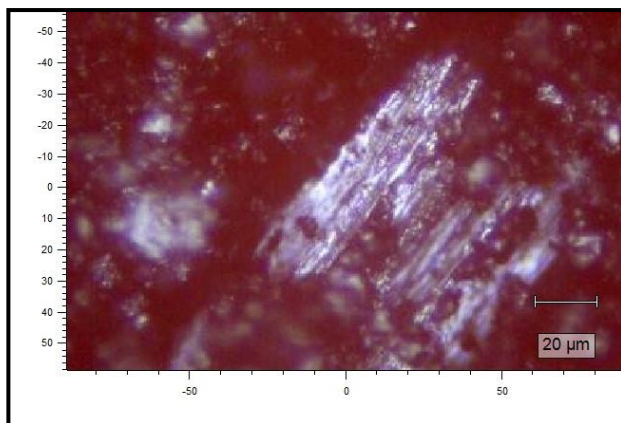
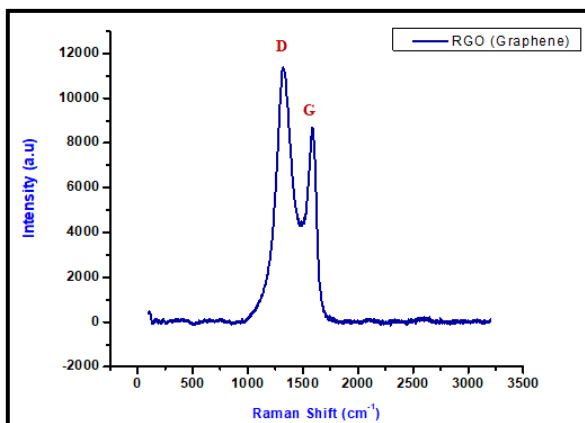


Figure 5.3: Raman spectroscopy results of rGO

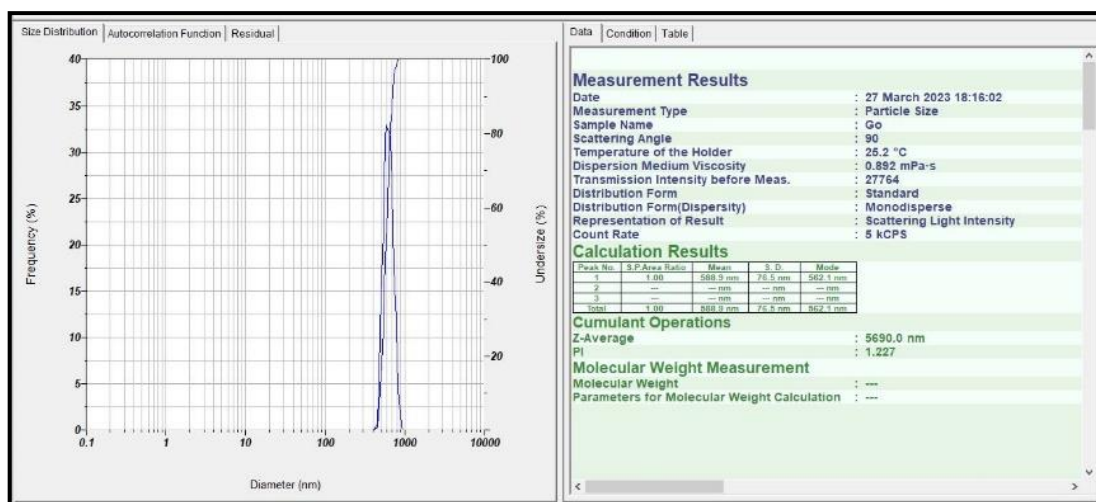


Figure 5.4: Zeta potential (particle size analysis) results of rGO

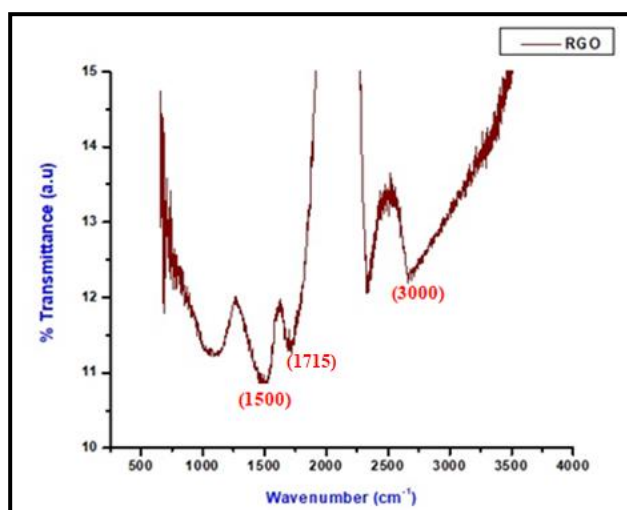


Figure 5.5: FTIR peaks of rGO

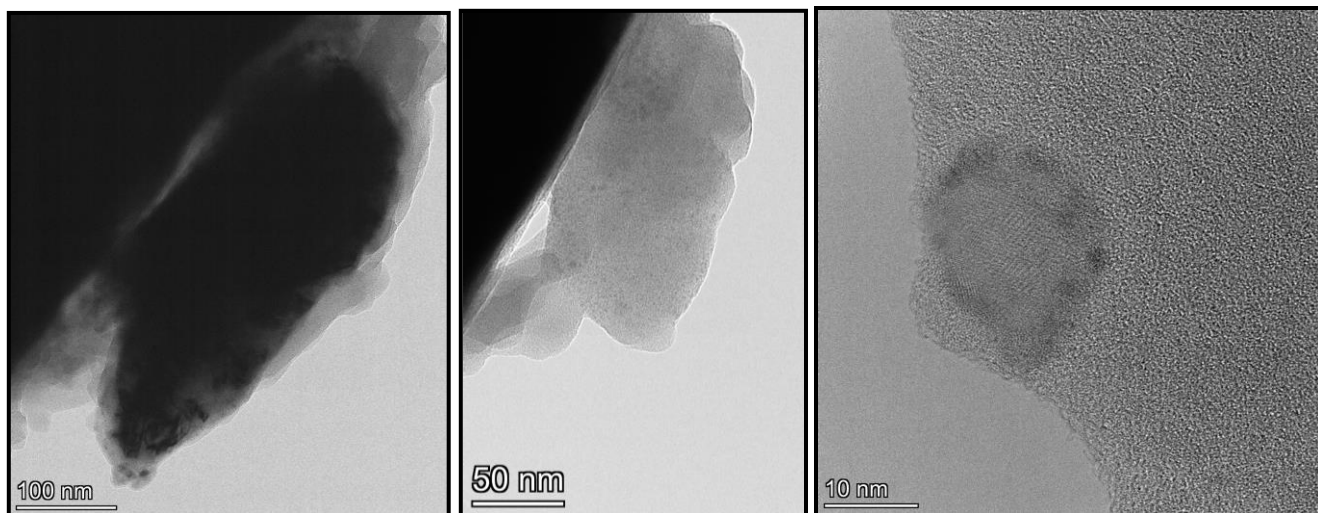


Figure 5.6: HR-TEM images of rGO

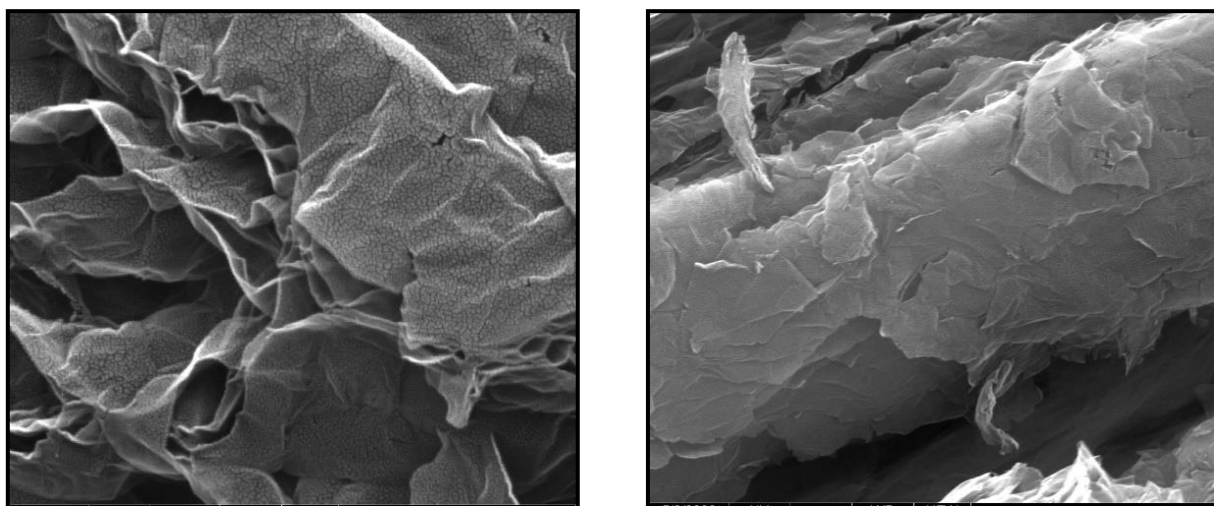
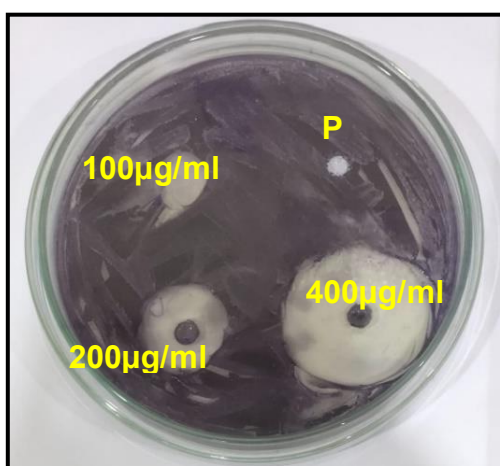


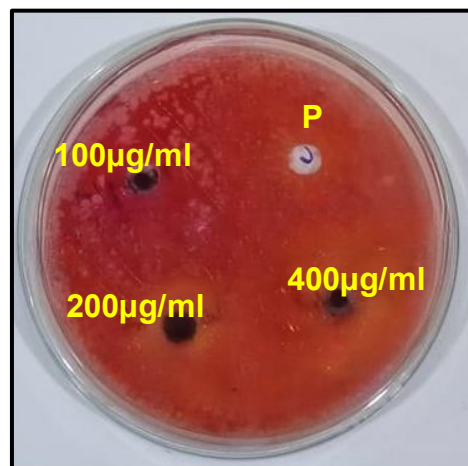
Figure 5.7: FE-SEM images of rGO

5.3 ANTIBACTERIAL ASSAY

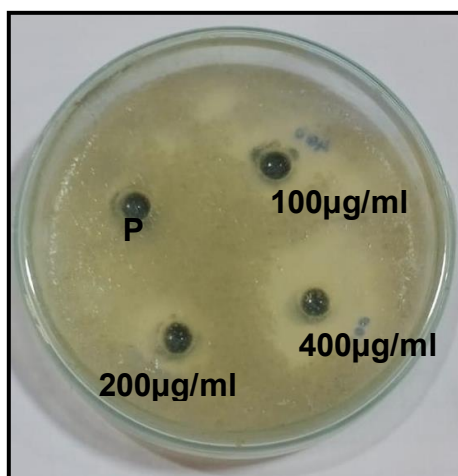
The antibacterial activities against *Staphylococcus aureus*, *Escherichia coli*, and *Proteus* were performed using MRGO graphene nanosheets, employing a well diffusion method, whose pictorial results are depicted in Figure 5.8 and tabulated ones in Table 5.1. *Proteus* showed the highest zone of inhibition in 400 $\mu\text{g/ml}$ (rGO powder dissolved in distilled water) of about 40mm and the other two bacteria *S.aureus* and *E.coli* depicted 27mm and 30mm respectively for 400 $\mu\text{g/ml}$ of rGO nanosheets.



(a)



(b)



(c)

Figure 5.8: Results of Antibacterial assay

(a) *Proteus_rGO*, (b) *E.coli_rGO*, (c) *S.Aureus_rGO*

Table 5.1: Antibacterial assay results

S.NO	BACTERIA	CONTROL: Penicillin	ZOI FOR 100µg/ml	ZOI FOR 200µg/ml	ZOI FOR 400µg/ml
1.	<i>Staphylococcus aureus</i>	7mm	8mm	15mm	27mm
2.	<i>Proteus</i>	6mm	10mm	20mm	40mm
3.	<i>Escherichia coli</i>	5mm	7mm	12mm	30mm

5.4 ANTI-FUNGAL ASSAY

Anti-fungal assay was tested against six different fungal phytopathogens, by uniformly mixing the prepared powder of rGO nanosheets in different concentrations in the sterilized PDA media. The results obtained on the 7th day after subculturing the fungus is represented below.

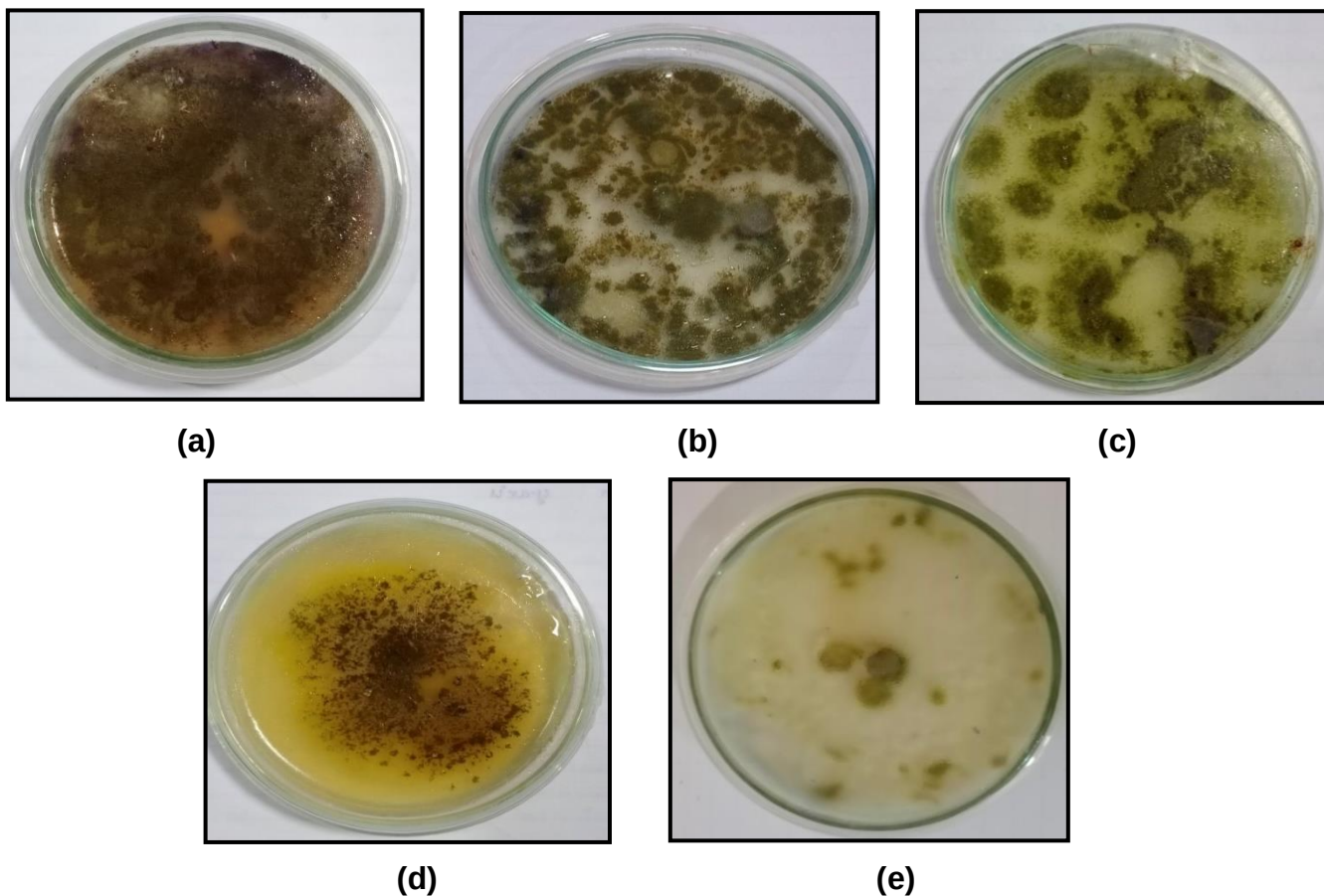
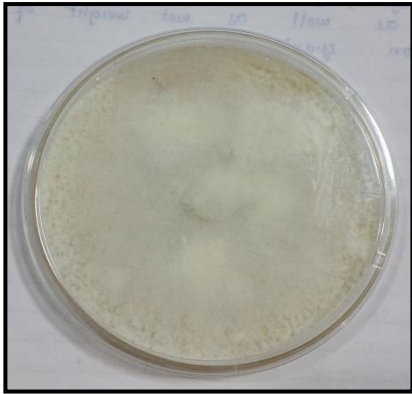


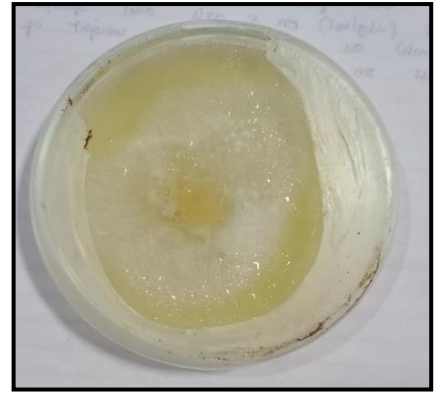
Figure 5.9: *Colletotrichum gloeosporioides*_rGO (a) Control, (b) 80 µg/20ml, (c) 150 µg/20ml, (d) 250 µg/20ml and (e) 500 µg/20ml



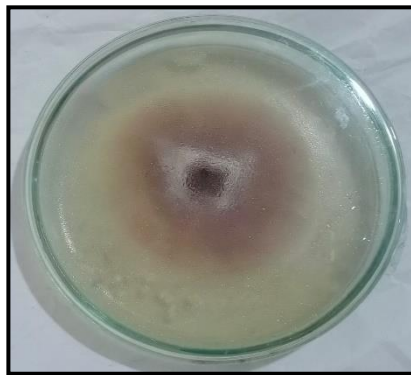
(a)



(b)



(c)



(d)



(e)

Figure 5.10: *Fusarium oryzae* f.sp. *cubense*_rGO (a) Control, (b) 80 μ g/20ml, (c) 150 μ g/20ml, (d) 250 μ g/20ml and (e) 500 μ g/20ml



(a)



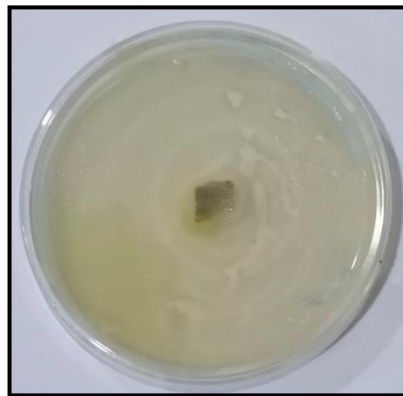
(b)



(c)



(d)



(e)

Figure 5.11: *Nigrospora oryzae*_rGO (a) Control, (b) 80 $\mu\text{g}/20\text{ml}$, (c) 150 $\mu\text{g}/20\text{ml}$, (d) 250 $\mu\text{g}/20\text{ml}$ and (e) 500 $\mu\text{g}/20\text{ml}$



(a)



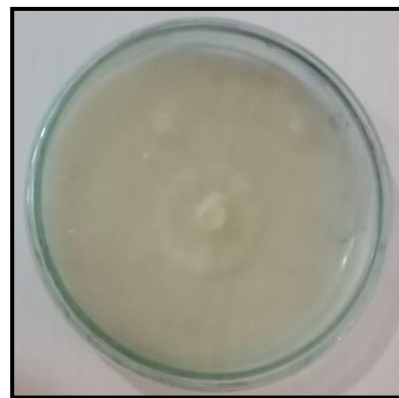
(b)



(c)



(d)



(e)

Figure 5.12: *Athelia rolfsii*_rGO (a) Control, (b) 80 $\mu\text{g}/20\text{ml}$, (c) 150 $\mu\text{g}/20\text{ml}$, (d) 250 $\mu\text{g}/20\text{ml}$, and (e) 600 $\mu\text{g}/20\text{ml}$

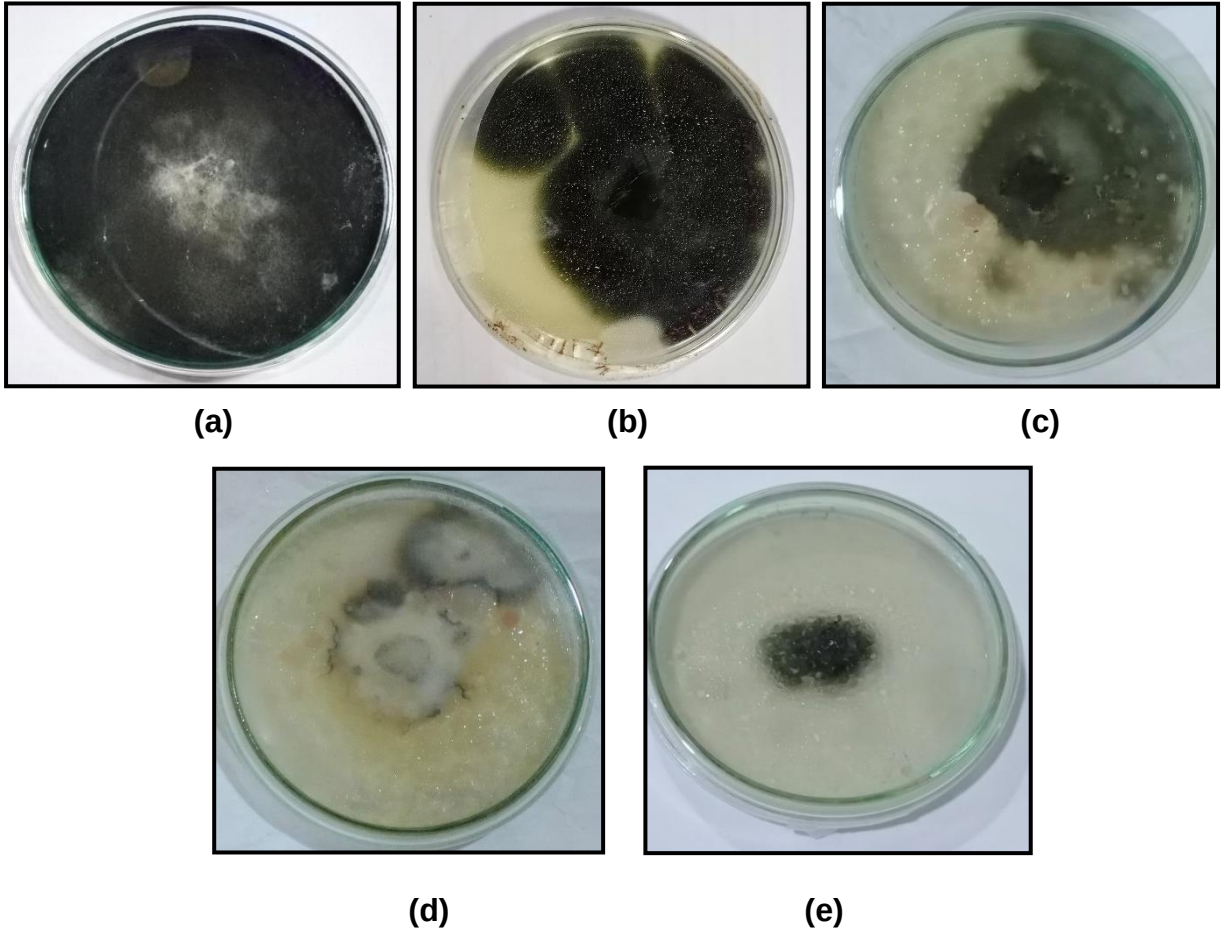
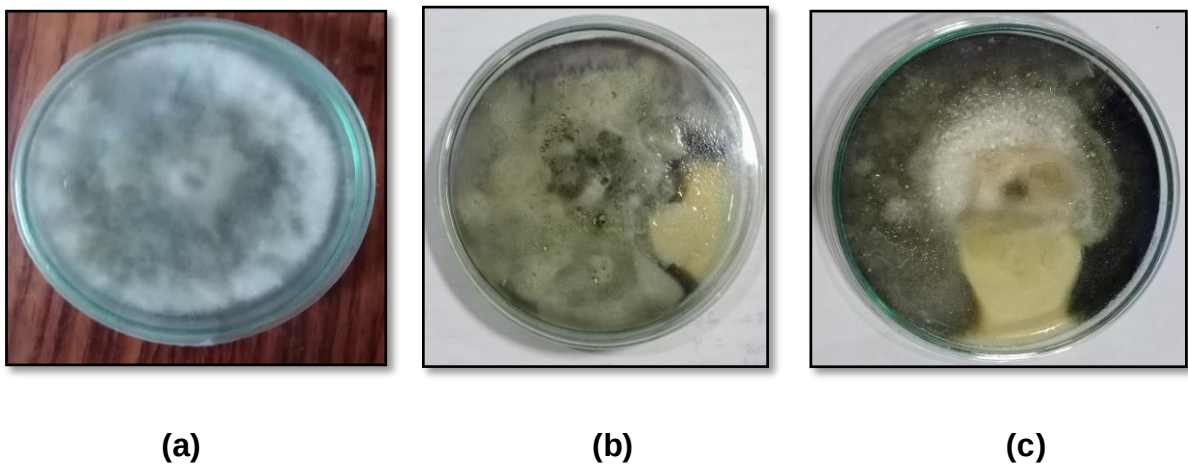
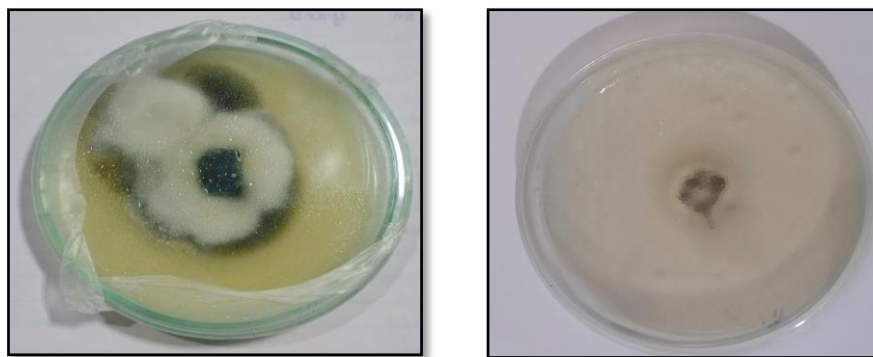


Figure 5.13: *Curvularia lunata_rGO* (a) Control, (b) 80 $\mu\text{g}/20\text{ml}$, (c) 150 $\mu\text{g}/20\text{ml}$, (d) 300 $\mu\text{g}/20\text{ml}$ and (e) 600 $\mu\text{g}/20\text{ml}$





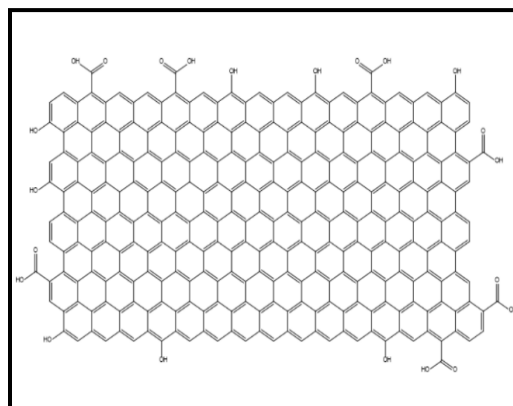
(d)

(e)

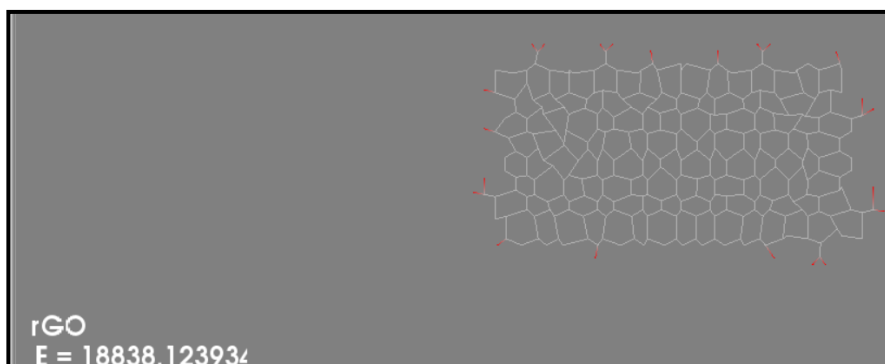
Figure 5.14: *Pyricularia oryzae*_rGO (a) Control, (b) 80 $\mu\text{g}/20\text{ml}$, (c) 150 $\mu\text{g}/20\text{ml}$, (d) 300 $\mu\text{g}/20\text{ml}$ and (e) 600 $\mu\text{g}/20\text{ml}$

5.5 IN-SILICO ANALYSIS

rGO nanosheet was created using Chemdraw software and it was used as both ligand and macromolecule to dock with specific proteins and drugs respectively for finding the toxicity pathway and the scope for rGO to act as drug carrier for targeted drug delivery binding affinity of the same. The results are tabulated in Table 5.2 and 5.3 with the pictorial depiction in Figure 5.23 and Figure 5.24.



(a)



(b)

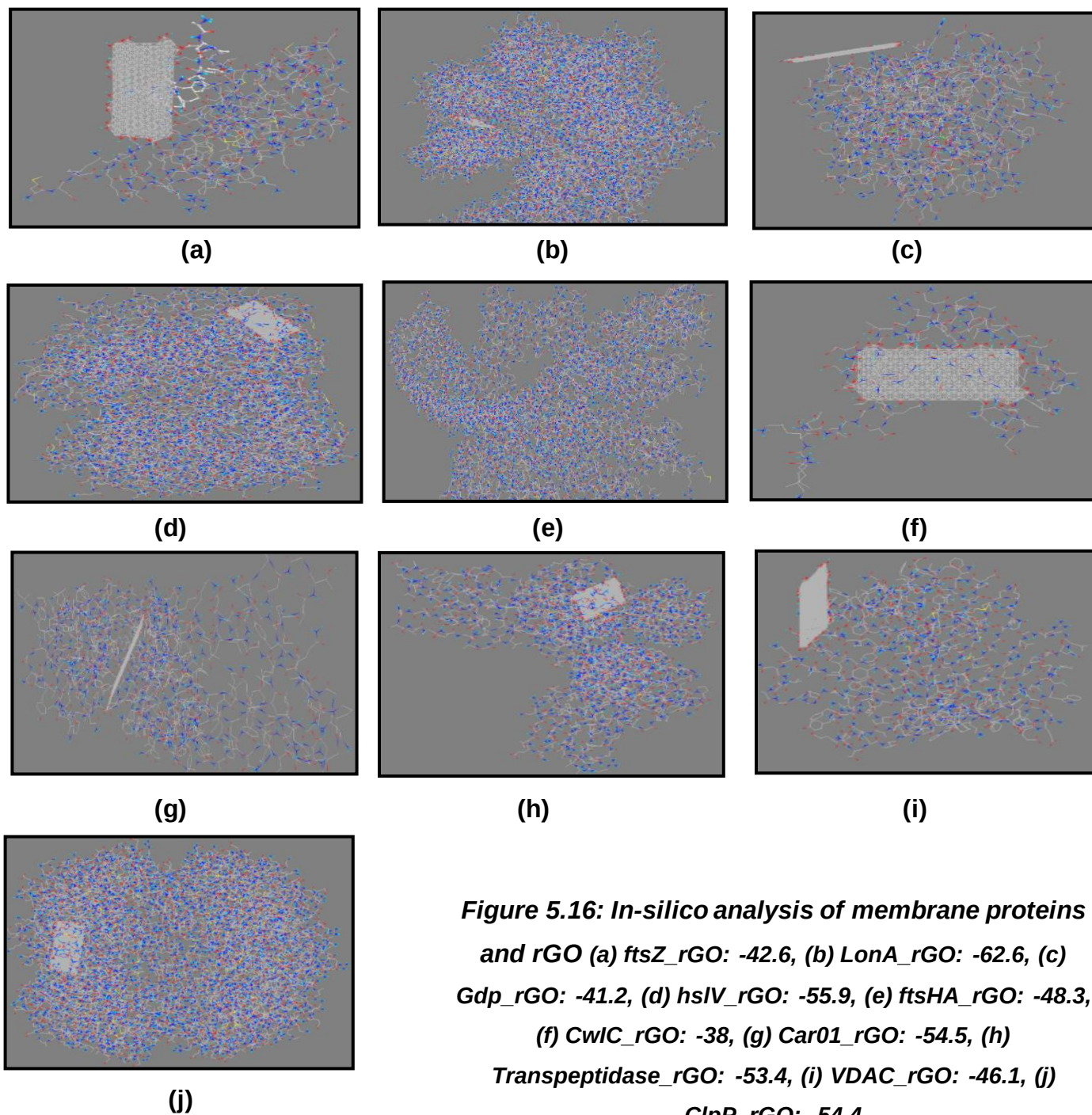
Figure 5.15: Preparing rGO nanosheets for in-silico docking analysis

(a) Construction of rGO nanosheets using chemdraw software

(b) Energy minimization of rGO

5.5.1 In-silico docking of membrane proteins and rGO

rGO nanosheets were docked with selected membrane proteins present in different bacteria to identify which protein has the highest binding affinity and to bring out the toxicity pathway by comparing these results with wet-lab analysis ones.



5.5.2 In-silico docking of DNA binding proteins and rGO

rGO nanosheets were docked with DNA binding membrane proteins present in different bacteria to check if the synthesised nanoparticle can interfere the central dogma of the prokaryotes. The results are depicted below in Figure 5.16.

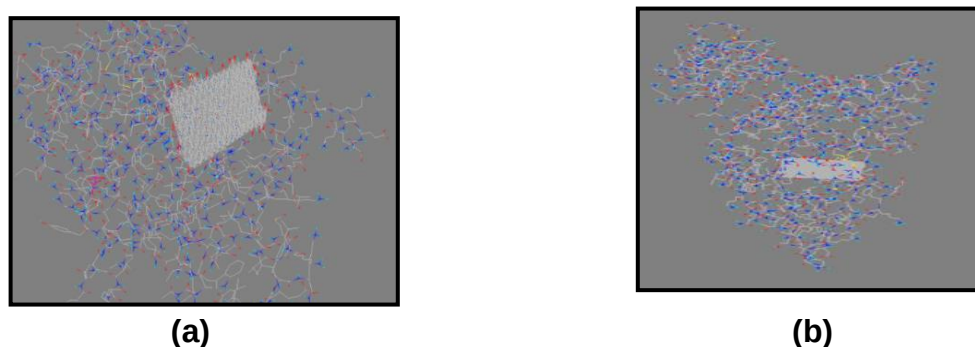


Figure 5.17: In-silico analysis of DNA binding proteins and rGO

(a) RecA_rGO: -45.9, (b) LexA_rGO: -47.1

5.5.3 In-silico docking of cancer related proteins and rGO

rGO nanosheets were docked with selected onco proteins of higher mammals to detect its anti-cancerous activity.

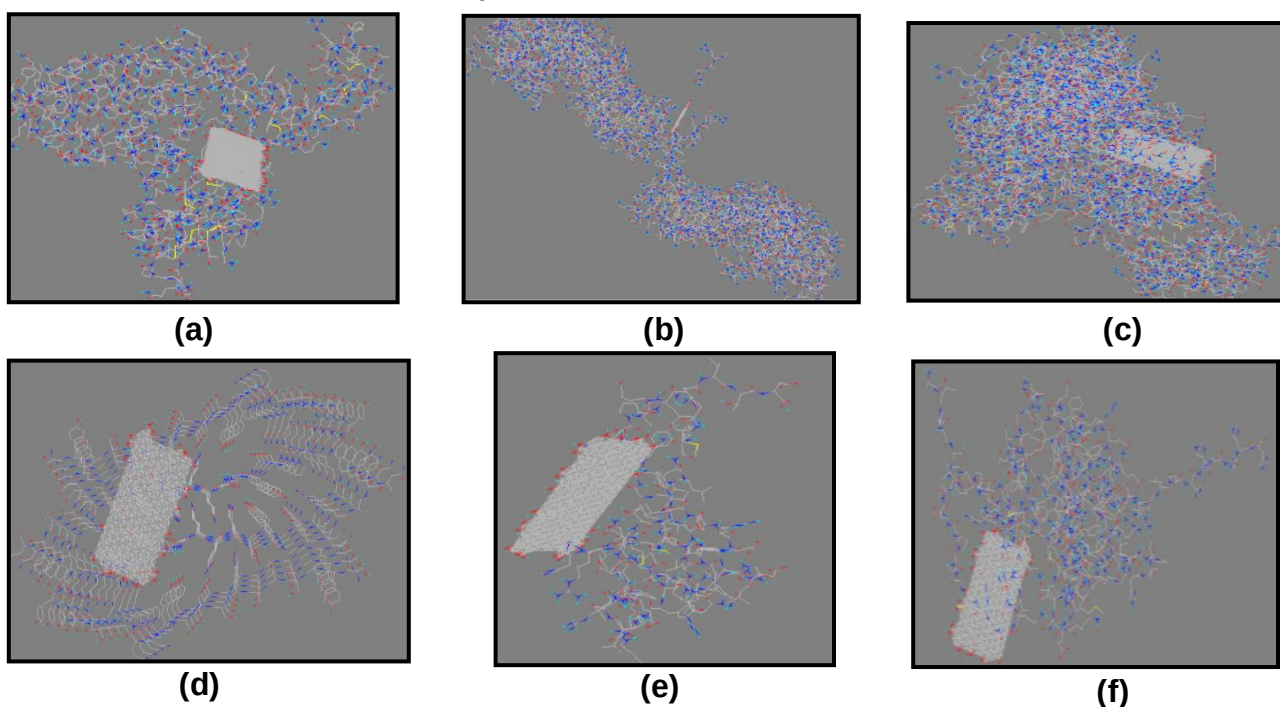
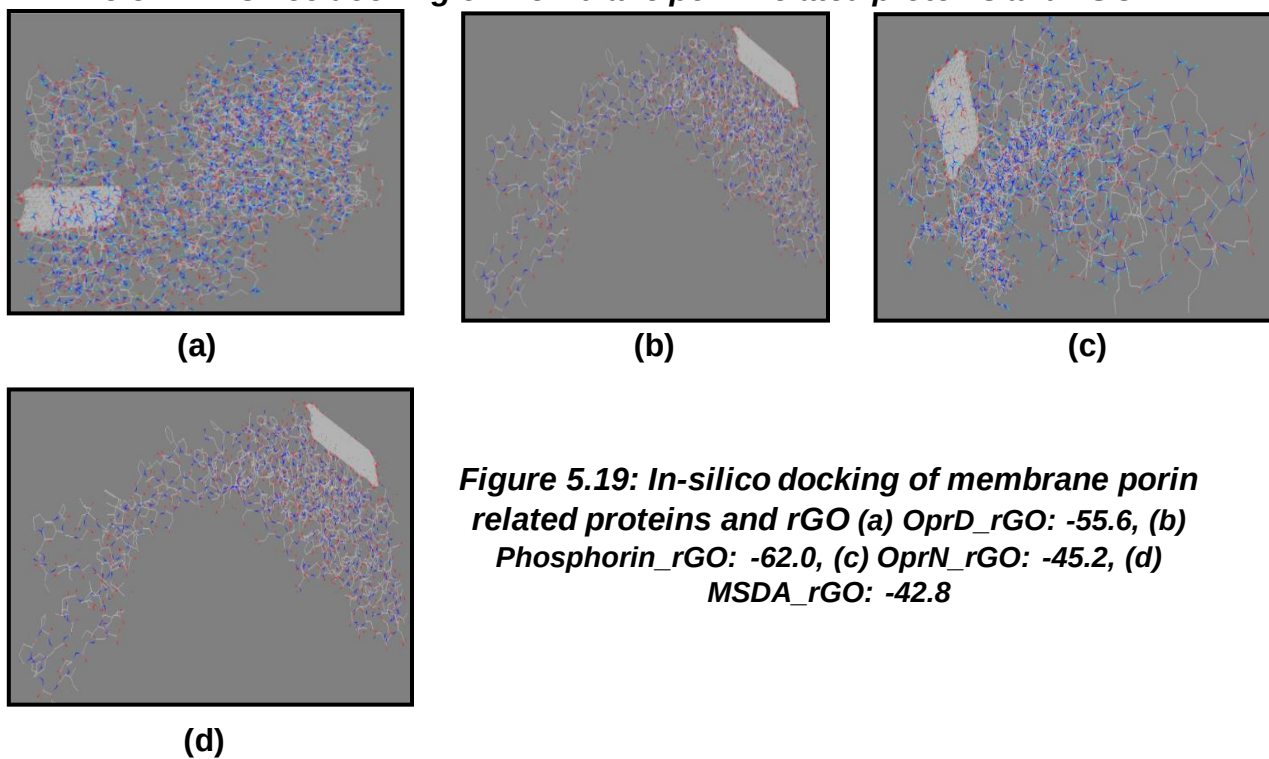


Figure 5.18: In-silico docking of cancer related proteins and rGO (a) TNF_rGO: -47.4, (b)

Menin_rGO: -46.7, (c) TP53_rGO: -50.6, (d) Lc_rGO: -56.8, (e) Caspase 7_rGO: -37.8, (f) BCL-W_rGO: -38.0

5.5.4 In-silico docking of membrane porin related proteins and rGO



5.5.5 In-silico docking of transport proteins and rGO

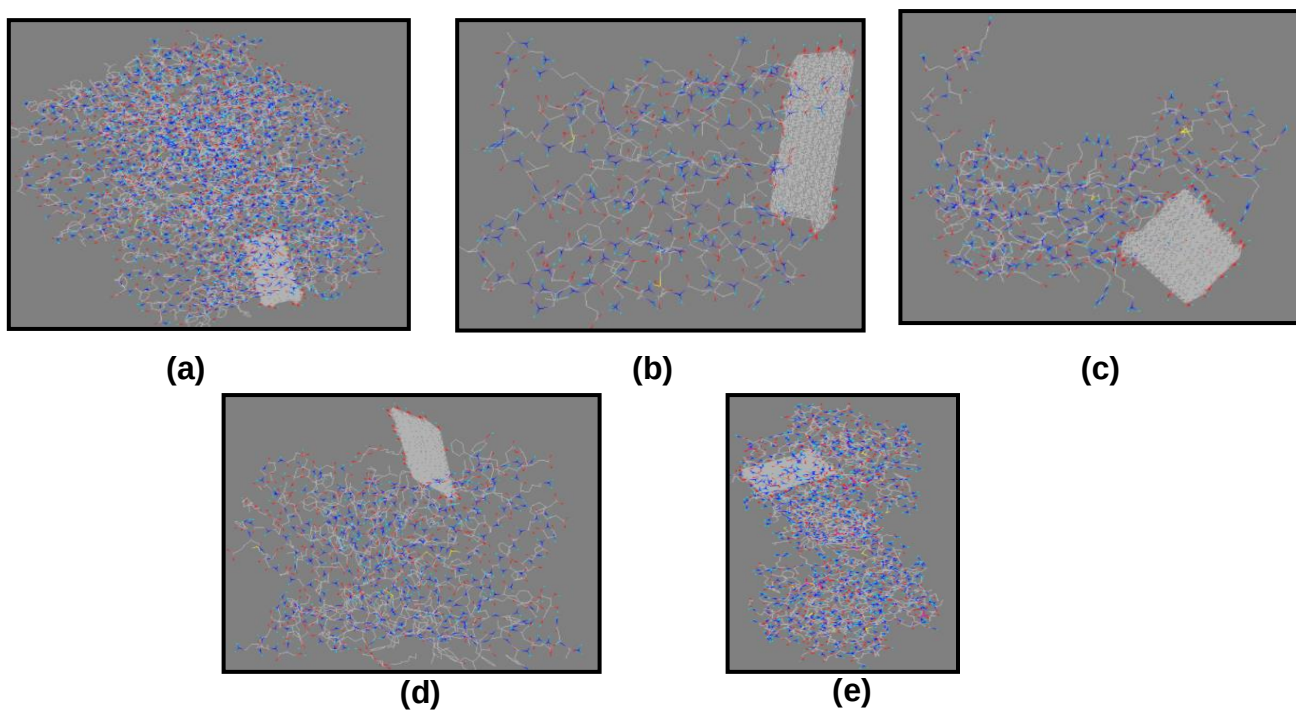


Figure 5.20: In-silico docking of transport proteins and rGO(a) OmpC_rGO: -49.9, (b) OmpATb_rGO: -40.2, (c) OmpA_rGO: -41.1, (d) OmpG_rGO: -57.7,(e)

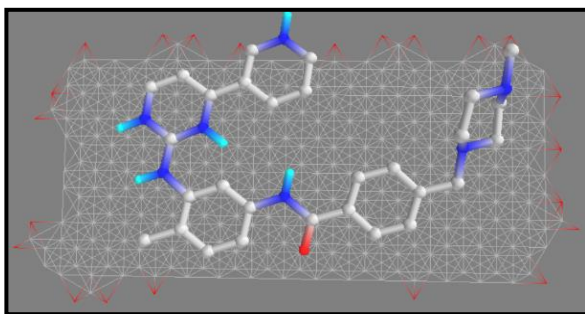
Table 5.2: Results of *In-silico* analysis of rGO nanosheet with specific proteins

S. No	Proteins	Classification	Role	Binding affinity(K _D)	PDB number	Organism
1	BclW	Cancer related protein	Anti-apoptotic protein, inhibition of this is helpful for cancer patients	-38	1MK3	<i>Homo sapiens</i>
2	Cas	Cancer related protein	Sequence specific RNA cleavage, control of transcription	-37.8	5O2M	<i>Homo sapiens</i>
3	Menin	Cancer related protein	Regulates gene transcription	-46.7	3U84	<i>Homo sapiens</i>
4	TNF	Cancer related protein	Inflammation-associated carcinogenesis	-47.4	1TNR	<i>Homo sapiens</i>
5	TP53	Cancer related protein	Tumor suppressor (regulates cell division)	-50.6	1XNI	<i>Homo sapiens</i>
6	Lc	Cancer related protein		-56.8	3WYD	Uncultured organism
7	Lex	DNA binding protein	Transcriptional repressor	-47.1	1JHF	<i>E. coli</i>
8	RecA	DNA binding protein	DNA repair in bacteria	-45.9	1XMV	<i>E. coli</i>
9	Car01	Membrane protein	Causing carbapenem resistance, loss of porins OmpK35 (outer membrane porin K35)	-54.5	4RL9	<i>Acinetobacter bumanii</i>

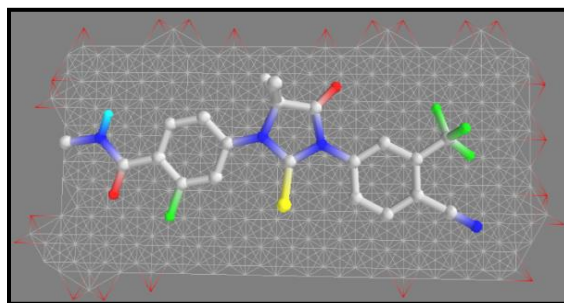
10	Cw1C	Membrane protein	Mother cell lysis	-38	1X60	<i>Bacillus subtilis</i>
11	C1pP	Membrane protein	Removal of damaged and unwanted protein	-54.4	IYG6	<i>E. coli</i>
12	FtsZ	Membrane protein	Cell wall synthesis	-42.6	1F47	<i>E. coli</i>
13	Gdp	Membrane protein	Binds to GPCR	-41.2	1RYA	<i>E. coli</i>
14	LonA	Membrane protein	Degrading proteins and proteolytic regulation	-62.6	6U5Z	<i>E. coli</i>
15	FtsH	Membrane protein	Degrades integral membrane proteins as well as cytoplasmic proteins	-48.3	7WI3	<i>E. coli</i> K-12
16	Hs1V	Membrane protein	Degraded certain hydrophobic peptides	-55.9	4G4E	<i>E. coli</i> K-12
17	Transpeptidase	Membrane protein	Catalyzes the nucleophilic carbonyl substitution process for the cross-linking	-53.4	1ZAT	<i>Enterococcus faecium</i>
18	VDAC	Membrane protein, Apoptosis	Regulates the mitochondrial porins	-46.1	2K4T	<i>Homo sapiens</i>
19	Phosphoporin	Porin	Pore formation in bilipid layer	-62	1PHO	<i>E. coli</i>

20	MspA	Porin	Major porin of <i>M. stegmatis</i>	-43.8	1UUN1	<i>Mycobacterium smegmatis</i>
21	OprD	Porin	Allows the entry of antibiotic into gram negative bacteria	-55.6	2ODJ	<i>Pseudomonas aeruginosa</i> PAO1
22	OprN	Porin	Allows the entry of antibiotic into gram negative bacteria	-45.2	5AZO	<i>Pseudomonas aeruginosa</i> PAO1
23	OmpAtb	Transport protein	For the bacteria to adapt in lower pH conditions and helps in survival of macrophages	-40.2	2KGS	<i>Mycobacterium tuberculosis</i>
24	OmpA	Transport protein	Bacterial adhesion, invasion	-41.1	2MQE	<i>E. coli</i> DH1
25	OmpC	Transport protein	Passive diffusion channels for (antibiotics)	-49.9	2J1N	<i>E. coli</i>
26	OmpG	Transport protein	Mediate the uptake of ions for growth of the cell	-57.7	2X9K	<i>E. coli</i> K-12
27	Atg	Transport protein	Autophagy-related	-50.8	6Y09	<i>Homo sapiens</i>

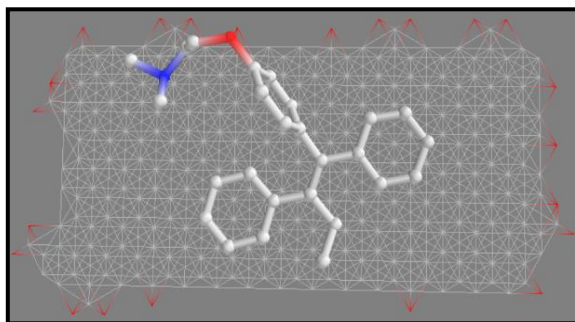
5.5.6 Molecular docking of rGO with specific drugs



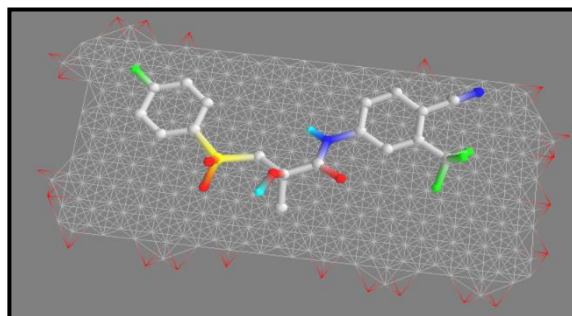
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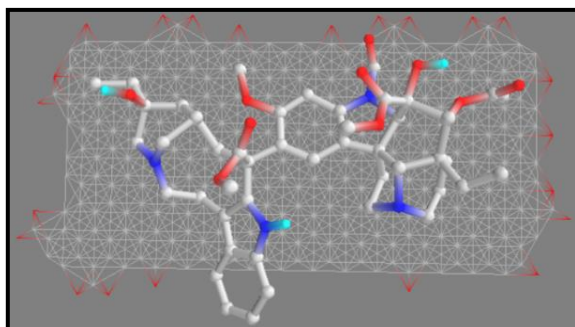
(b)



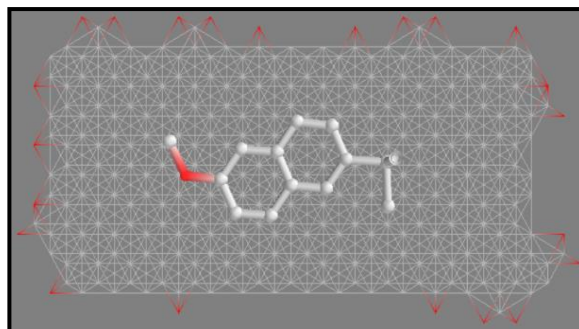
(c)



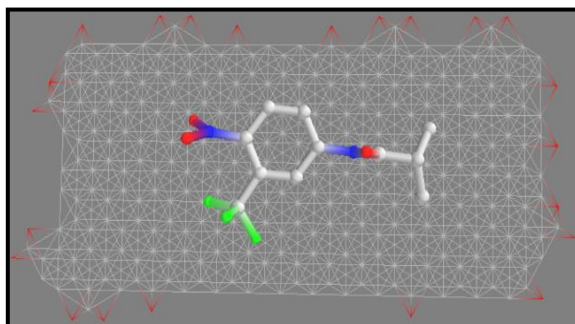
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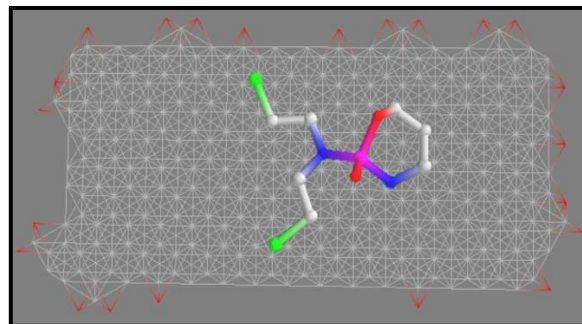
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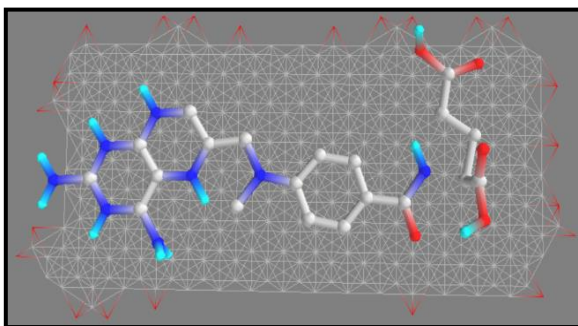
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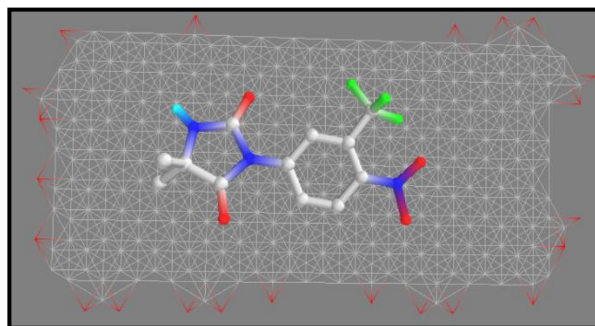
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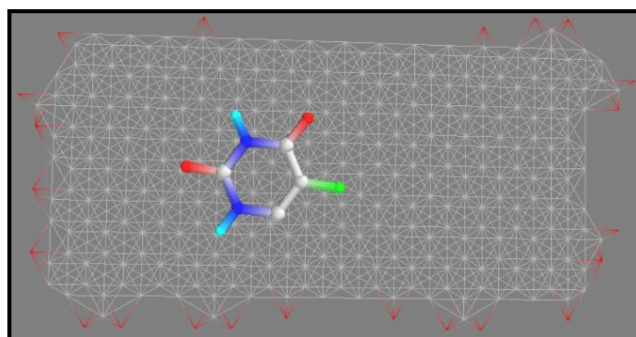
(h)



(i)



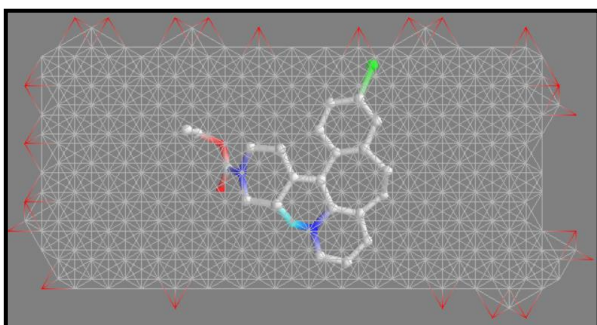
(j)



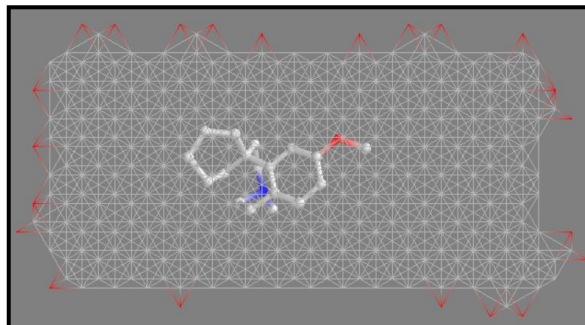
(k)

Figure 5.21: Anti-cancer drugs docked with rGO

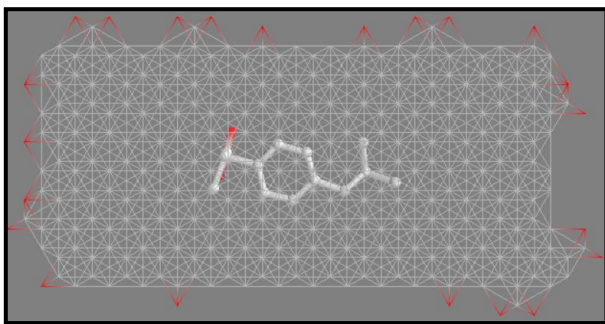
(a) Imatinib_rGO: -39.6, (b) Xtandi_rGO: -36.7, (c) Tamoxifen_rGO: -32, (d) Casodex_rGO: -31, (e) Vincristine: -27.6, (f) Naproxen_rGO: -26.3, (g) Methotrexate_rGO: -26, (h) Anandron_rGO: -24.9, (i) Flutamide_rGO: -22.4, (j) Cyclophosphamide_rGO: -13.1, (k) Carac_rGO: -10.6



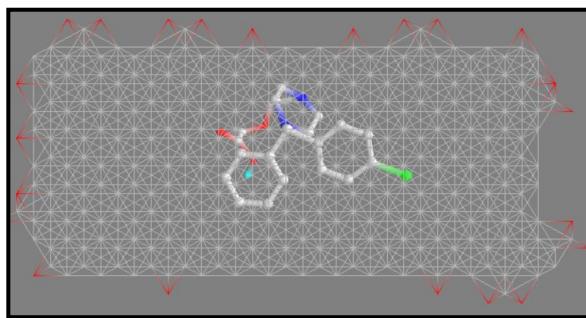
(a)



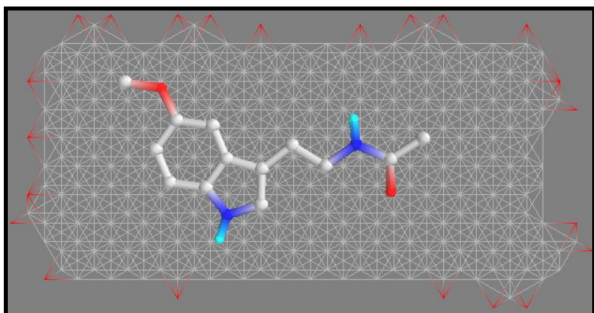
(b)



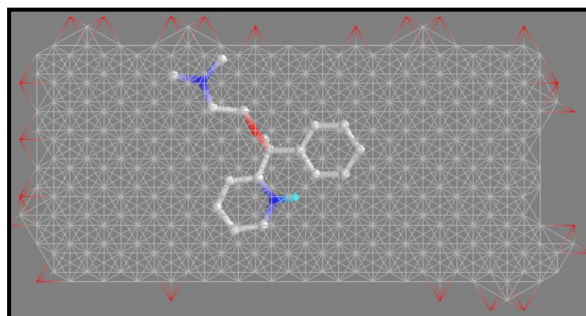
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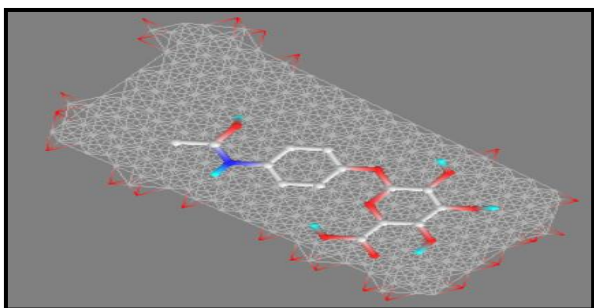
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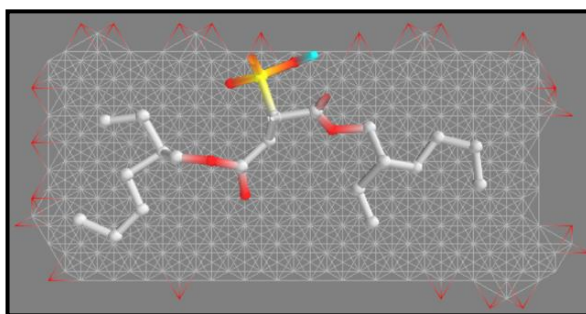
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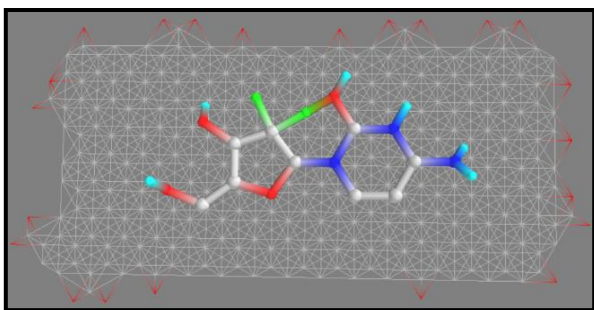
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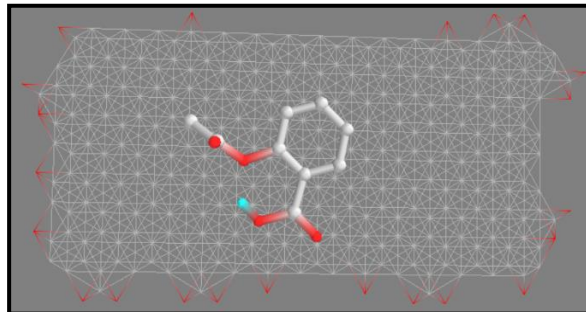
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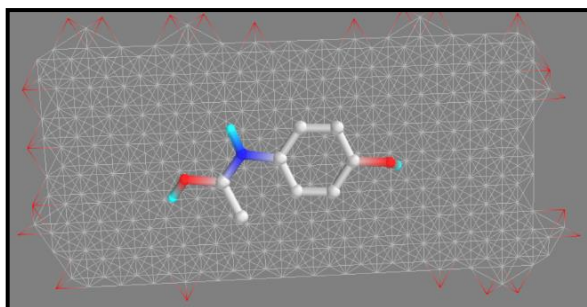
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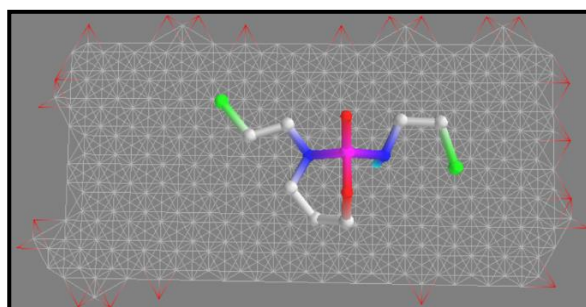
(i)



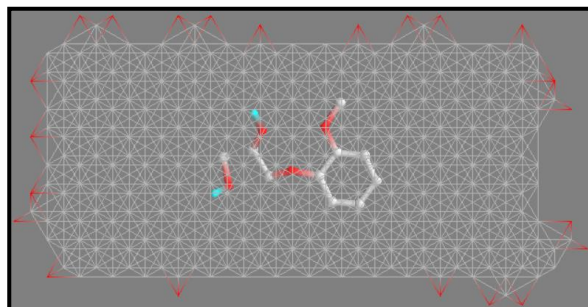
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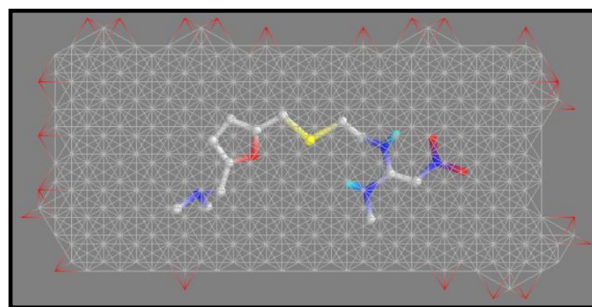
(k)



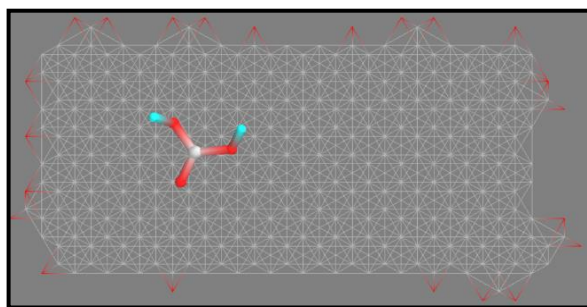
(l)



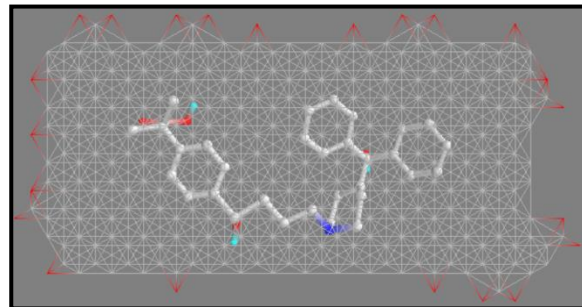
(m)



(n)



(o)



(p)

Figure 5.22: OTC drugs docked with rGO

(a) Loratadine_rGO: -34.4, (b) Dxm_rGO: -25.6, (c) Ibuprofen: -23.6, (d) Cetirizine_rGO: -22.7, (e) Melatonin_rGO: -22.5, (f) Doxylamine_rGO: 22.1, (g) Paracetamol_glucuronide_rGO: -21.5, (h) Docusate_rGO: -20.7, (i) Gemcitabine_rGO: -19.6, (j) Aspirin_rGO: -17.5, (k) Acetaminophen_rGO: -16.8, (l) Rantidine_rGO: -14.9, (m) Tussin_rGO: -14.6, (n) Ifosfamide_rGO: -13.2, (o) Carbonate: -4.2, (p) Fexofenadine_rGO: -31.7

Table 5.3: Results of molecular docking of rGO with different anti-cancer and over-the-counter drugs

S. No	Ligand	Name of the drug	Role of the drug	Drug type	Molecular formula	Binding Affinity(K _D)
1	ZINC19632618	Imatinib	Treats leukemia	Anti-cancer	C ₂₉ H ₃₁ N ₇ O	-39.6
2	ZINC34806477	Xtandi	Treats prostate cancer	Anti-cancer	C ₂₁ H ₁₆ F ₄ N ₄ O ₂ S	-36.7
3	ZINC01530689	Tamoxifen	Treats breast cancer in men and women	Anti-cancer	C ₂₆ H ₂₉ NO	-32
4	ZINC00538564	Casodex	Treats for prostate cancer.	Anti-cancer	C ₁₈ H ₁₄ F ₄ N ₂ O ₄ S	-31
5	ZINC85432549	Vincristine	Blocks cell growth by stopping mitosis.	Anti-cancer	C ₄₆ H ₅₆ N ₄ O ₁₀	-27.6
6	ZINC00105216	Naproxen	treating acute gout and various inflammations.	Anti-cancer	C ₁₄ H ₁₄ O ₃	-26.3
7	ZINC01529323	Methotrexate	Treats cancer by slowing the growth of cancer cells	Anti-cancer	C ₂₀ H ₂₂ N ₈ O ₅	-26
8	ZINC03874498	Anandron	Blocks testosterone receptors	Anti-cancer	C ₁₂ H ₁₀ F ₃ N ₃ O ₄	-24.9
9	ZINC03812944	Flutamide	Treats treatment of androgen-dependent tumors like prostate cancer and PCOS	Anti-cancer	C ₁₁ H ₁₁ F ₃ N ₂ O ₃	-22.4
10	ZINC00155694	Cyclophosphamide	Treats cancer.	Anti-cancer	C ₇ H ₁₅ Cl ₂ N ₂ O ₂ P	-13.1

11	ZINC38212689	Carac	Treats skin cancer.	Anti-cancer	C ₄ H ₃ FN ₂ O ₂	-10.6
12	ZINC00537931	Loratadine	Relieve and treat allergic rhinitis and urticaria.	OTC	C ₂₂ H ₂₃ ClN ₂ O ₂	-34.4
13	ZINC03201907	Dxm	Temporarily relieves cough caused by the common cold, the flu, or other conditions.	OTC	C ₁₈ H ₂₅ NO	-25.6
14	ZINC00002647	Ibuprofen	Manage and treats inflammatory diseases.	OTC	C ₁₃ H ₁₈ O ₂	-23.6
15	ZINC19364229	Cetirizine	An antihistamine medicine that helps the symptoms of allergies.	OTC	C ₂₁ H ₂₅ ClN ₂ O ₃	-22.7
16	ZINC00057060	Melatonin	Treats delayed sleep phase and circadian rhythm	OTC	C ₁₃ H ₁₆ N ₂ O ₂	-22.5
17	ZINC00000242	Doxylamine	Manage and treats nausea and vomiting of pregnancy.	OTC	C ₁₇ H ₂₂ N ₂ O	-22.1
18	ZINC05197198	Paracetamol Glucuronide	Widely used analgesic and antipyretic agent	OTC	C ₁₄ H ₁₇ NO ₈	-21.5
19	ZINC04097298	Docusate	Management and treatment of constipation.	OTC	C ₂₀ H ₃₈ O ₇ S	-20.7
20	ZINC18279854	Gemcitabine	Stops the growth of cancer	OTC	C ₉ H ₁₁ F ₂ N ₃ O ₄	-19.6

			cells in your body.			
21	ZINC02382375	Paracetamol sulfate	Relief of pain as an antipyretic.	OTC	$C_8H_9NO_5S$	-19.1
22	ZINC00000053	Aspirin	Stops the production of certain natural substances that causes fever, pain, swelling, and blood clots.	OTC	$C_9H_8O_4$	-17.5
23	ZINC13550868	Acetaminophen	A non-opioid analgesic and antipyretic agent	OTC	$C_8H_9NO_2$	-16.8
24	ZINC01530729	Ranitidine	Works by reducing the amount of acid your stomach produces.	OTC	$C_{13}H_{22}N_4O_3S$	-14.9
25	ZINC00000353	Tussin	Treats cough and chest congestion.	OTC	$C_{10}H_{14}O_4$	-14.6
26	ZINC01530659	Ifosfamide	Treats various cancers.	OTC	$C_7H_{15}Cl_2N_2O_2P$	-13.3
27	ZINC06827693	Carbonate	Used as an antacid to relieve heartburn.	OTC	CH_2O_3	-4.2
28	ZINC03824921	Fexofenadine	Helps with allergies.	OTC	$C_{32}H_{39}NO_4$	-31.7

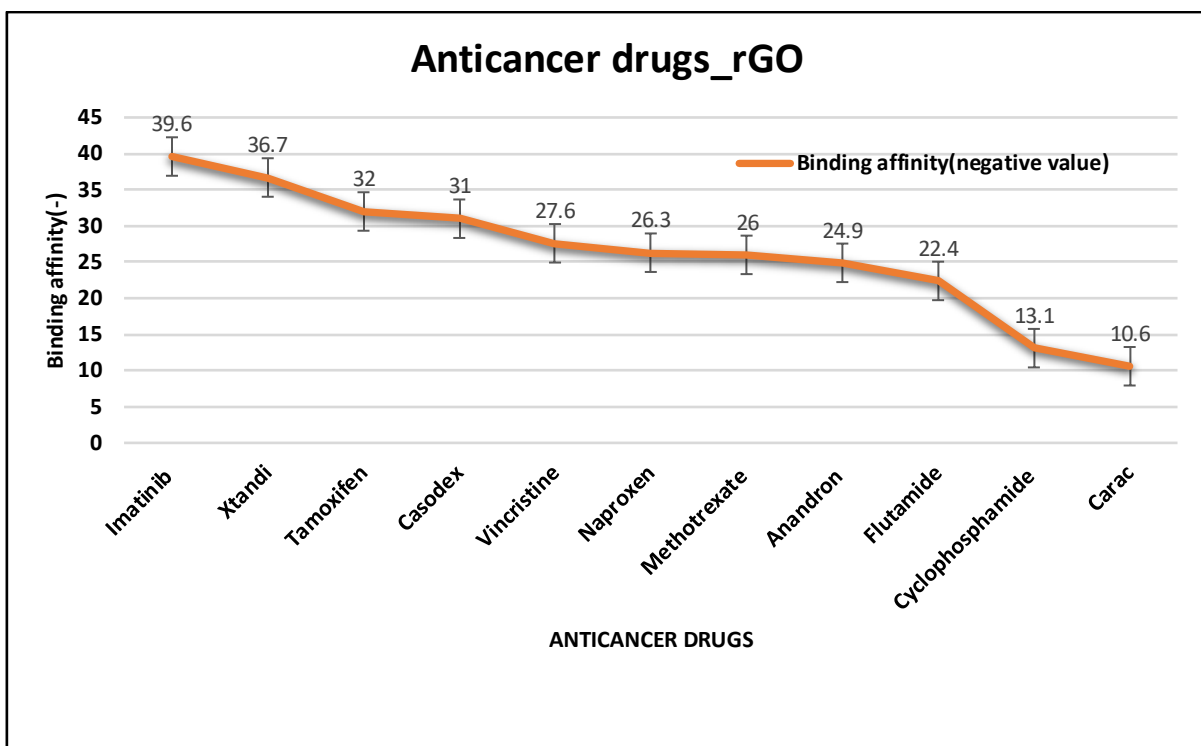


Figure 5.23: Binding affinity of Anticancer drugs docked with rGO

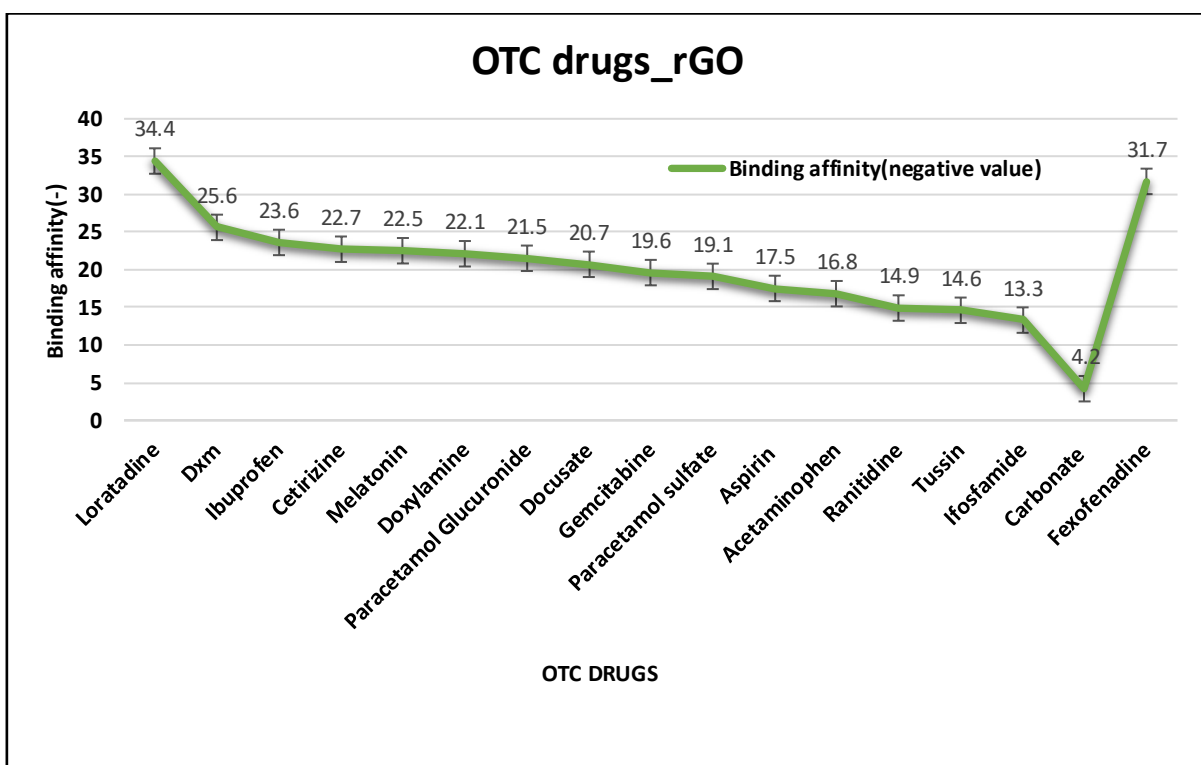


Figure 5.24: Binding affinity of Over the Counter drugs docked with rGO

6. CONCLUSION

Even while it currently only accounts for a tiny percentage of total research on these ground-breaking 2D materials, the study of the graphene family of nanoparticles (GFNs) and how they interact with biosystems is expanding quickly. Graphene belongs to the family of nanomaterials because of its surface chemistry, lateral dimension, and several layers. These changes are likely to have an impact on biological response. This review paper examines the electrical and structural characteristics of graphene and discusses current findings in graphene-based biocompatibility. GO and rGO exhibit various mechanical, electrical, and chemical capabilities because of their disparate chemical and structural compositions. rGO nanosheets are very much toxic to both bacterial and fungal species, among the three species of bacteria used, *Proteus* shows the larger zone of inhibition of about 40mm in 400µg/ml, which is followed by *E. coli* of 30mm ZOI and *S. aureus* of 7mm. There was the exploitation of seven fungal phytopathogens to test the antifungal effect, in which *Colletotrichum gloeosporioides*, *Fusarium oxysporum f.sp. Cubense* and *Nigrospora oryzae* growth was completely inhibited at 500µg/20ml. On the other hand, phytopathogen whose growth was inhibited at 600µg/20ml was *Athelia rolfsii*. *Pyricularia oryzae* and *Curvularia lunata* had their growth inhibited at 700µg/20ml.

There was also *in-silico* analysis performed by docking rGO with few specific proteins and with few anti-cancer and over-the-counter drugs. LonA, which is a membrane protein has the highest binding affinity among the proteins docked with an rGO nanosheet of about -62.6, and the second highest is Phosphorin with -62, which is a porin, these results suggest that the rGO nanosheet can perfectly bind with the membranous proteins of bacteria and elicit the toxicity pathway, this proves it be the efficient antibiotic material. Imatinib, an anti-cancer drug proves to be having high binding affinity of about -39.6, and Loratadine with a binding affinity of -34.4 has the highest binding affinity among the docked over-the-counter drug, this proves that rGO nanosheets can also be used perfectly for targeted drug delivery applications.

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ANNEXURE

1. Unravelling the ubiquitous role of mysterious graphene material over the prospective biological systems: A review

Future Journal of Pharmaceutical Sciences	
Unravelling the ubiquitous role of mysterious graphene material over the prospective biological systems: A review --Manuscript Draft--	
Manuscript Number:	
Full Title:	Unravelling the ubiquitous role of mysterious graphene material over the prospective biological systems: A review
Article Type:	Review
Funding Information:	
Abstract:	Abstract Background: Graphene is the most notable substance among the newly found and created nanostructured carbonaceous species. The extraordinary features of graphene (electronic conductivity, mechanical resilience, enormous surface area) have caused a profound shift in the field of material research. In recent years, graphene and its derivative composite materials have dived into various fields of technology. However, varied physicochemical qualities resulted in an extraordinary impact on its biological response. The biocompatibility of this nanomaterial over different biological systems is a developing research topic. Graphene, having an irregular surface, elicits cidal activity against various organisms depending upon the concentration imparted. With its toxicological investigations and physiological views on various biological systems including prokaryotes, eukaryotes, and a few chosen higher-level creatures, this review article focuses on the many underlying features of graphene. Result: The study would enable us to uncover several deeper insights about the Graphene material and its potential implications while devising therapeutic and biomedical agents while dealing with complex biological systems. Conclusion: This research compiled some fascinating characteristics of graphene-based nanomaterials in biological systems. It emphasized the most current developments in research on the production and study of graphene and its derivatives, the extracellular interactions of graphene with biomacromolecules, the cytotoxicity of graphene, the toxicological effects of graphene in vivo.

Participated and presented the poster at ICONN conference.





UNCOVERING THE TOXICOLOGICAL PROPERTIES OF REDUCED GRAPHENE OXIDE (rGO) SHEETS OVER PYRICULARIA ORYZAE.

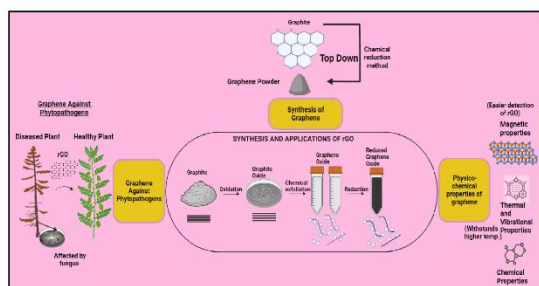
Rakshi Anuja Dinesh, Jayashree Shanmugam and Kunal Biswas.

Presenting author: Rakshi Anuja Dinesh, rakshising@gmail.com.

Corresponding author: Kunal Biswas, kunalbiswas.irc@sathyabama.ac.in.

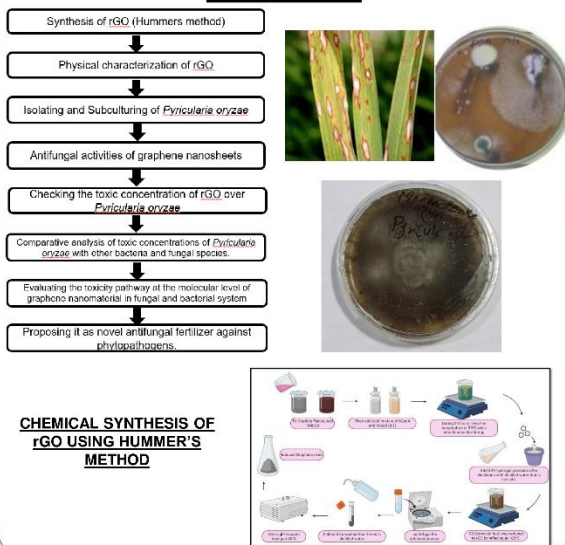


Introduction



- Fungal phytopathogens varying to become resistant to synthetic pesticides and antagonist micro-organisms are causing a huge loss in agricultural production (Lahlali R et al., 2022).
- A fungal species that causes rice blast is *Pyricularia oryzae*. It is a fast-growing pathogen that is resistant to several synthetic and bio methods of controlling them (Qi, H. et al., 2019).
- Nanotechnology is an evolving field, where it uses nanomaterials for solving many problems in this fast-paced world. Graphene is one such nanomaterial that has a rough surface and is irregular according to its SEM image because of which it can disrupt the fungal cell. Along with this, its physicochemical properties will enhance its application (Priyadarsini Subhashree et al., 2018).
- With the toxicological investigations of fungus, graphene family nanoparticles, especially reduced graphene oxide can be developed as a nano-fertilizer against rapidly growing fungal phytopathogens.
- This study aims at finding the exact toxic concentration of rGO over the same which will help us devise a potential fertilizer against other resistant fungi.
- The study will also uncover the toxicity pathway of rGO over the same which will help us devise a potential fertilizer against other resistant fungi.

Methods

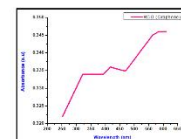


Results

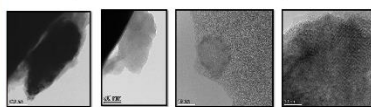
Phytopathogen confirmation:
microscopic image of *Pyricularia oryzae*



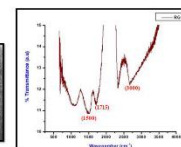
UV Vis results of rGO



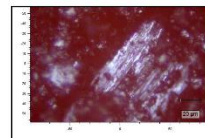
TEM images of rGO



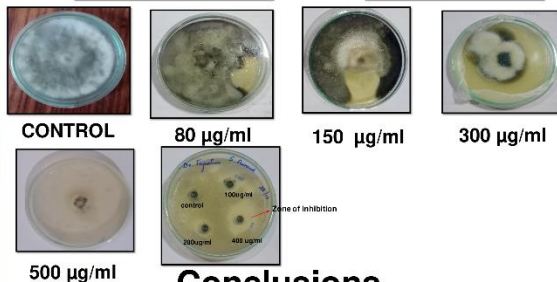
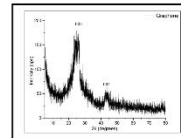
FTIR of rGO



XRD results of rGO.



Raman spec



Conclusions

- At 500 µg/ml of reduced graphene oxide (rGO) there is complete inhibition of growth of *Pyricularia oryzae*.
- At 400 µg/ml of reduced graphene oxide (rGO) there is increased diameter of zone of inhibition.

Acknowledgment

The authors thank the Department of Biotechnology, Sathyabama Institute of Science and Technology, and International Research Centre, Centre for Nanoscience and Nanotechnology, Sathyabama Institute of Science and Technology for carrying out the work.

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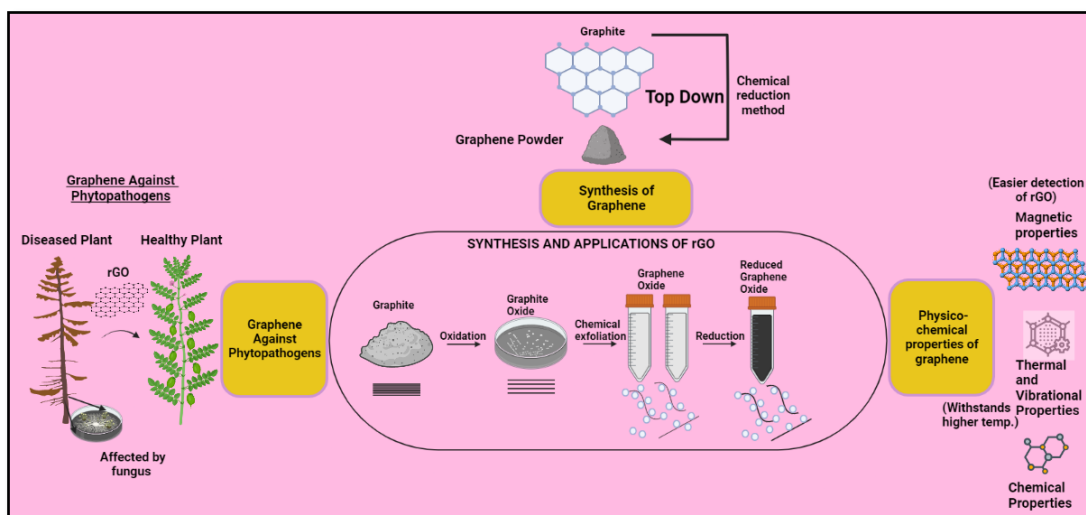
UNCOVERING THE TOXICOLOGICAL PROPERTIES OF REDUCED GRAPHENE OXIDE (rGO) OVER *Pyricularia oryzae*.

Rakshi Anuja Dinesh, Jayashree Shanmugam and Kunal Biswas

In recent years, fungal phytopathogens varying to become resistant to synthetic pesticides and antagonist micro-organisms are causing a huge loss in agricultural production. A fungal species that cause disease in rice crop plants is *Pyricularia oryzae*, it is a fast-growing pathogen that is resistant to several synthetic and bio methods of controlling them. Nanotechnology is an evolving field, where it uses nanomaterials for solving many problems in this fast-paced world. Graphene is one such nanomaterial that has a rough surface and is irregular according to its SEM image because of which it can disrupt the fungal cell wall. Along with this, its physicochemical properties will enhance its application. With the toxicological investigations of fungus, graphene family nanoparticles, especially reduced graphene oxide can be developed as a nano-fertilizer against rapidly growing fungal phytopathogens. This study aims at finding the exact toxic concentration from 5 µg/ml to 100 µg/ml of reduced graphene oxide over the fungal phytopathogens. The study will also uncover the toxicity pathway of rGO over the same which will help us devise a potential fertilizer against other resistant fungi.

Keywords: *Reduced graphene oxide, toxicology, Pyricularia oryzae, physicochemical properties, graphene, fungal phytopathogens.*

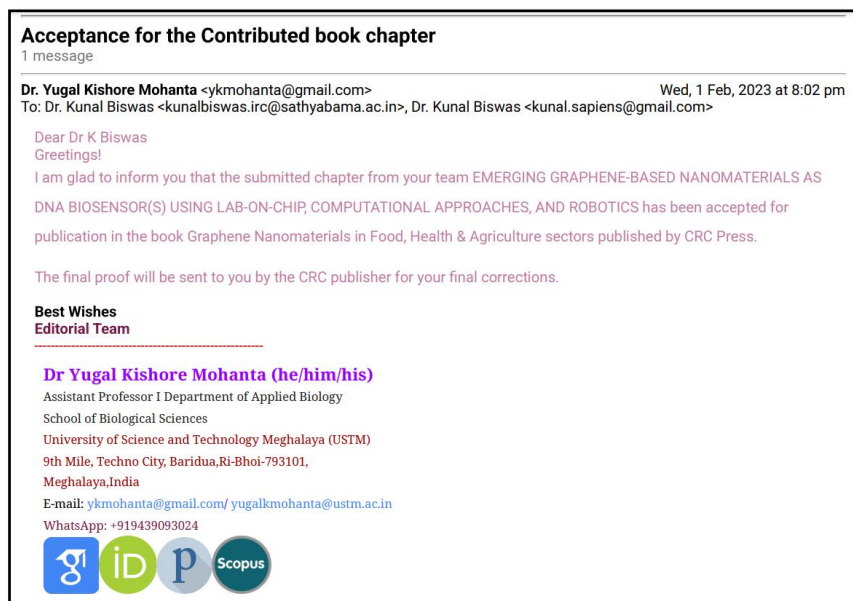
GRAPHICAL ABSTRACT



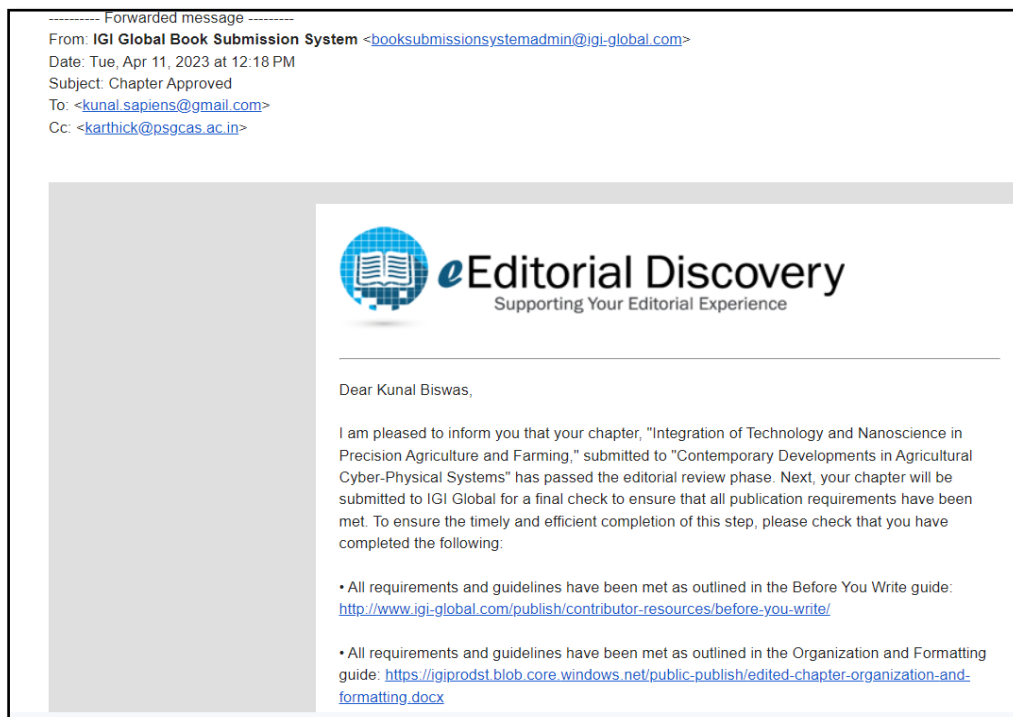
BOOK CHAPTERS

1. Emerging graphene-Based Nanomaterials as DNA biosensors(s) using Lab-on-chip, computational approaches and robotics: Book Chapter accepted

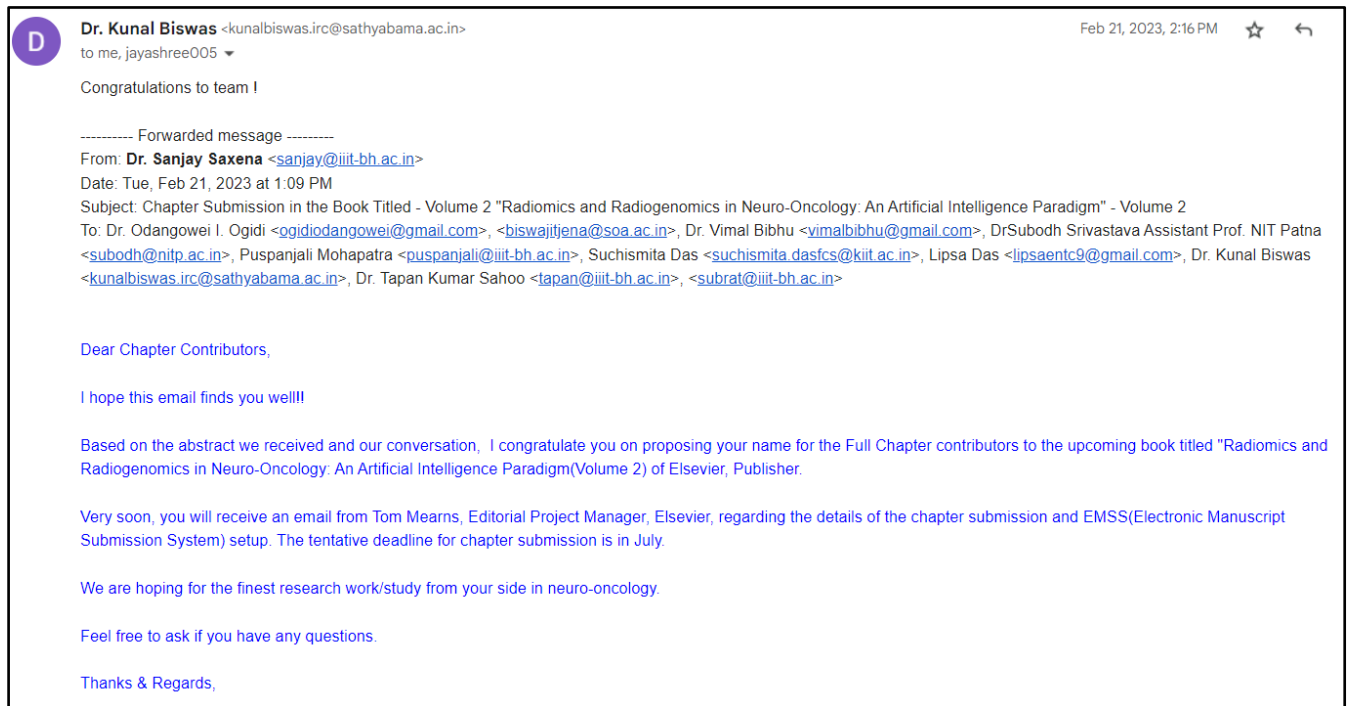
Accepted in Elsevier, CRC press (Taylor & Francis Group) Journal



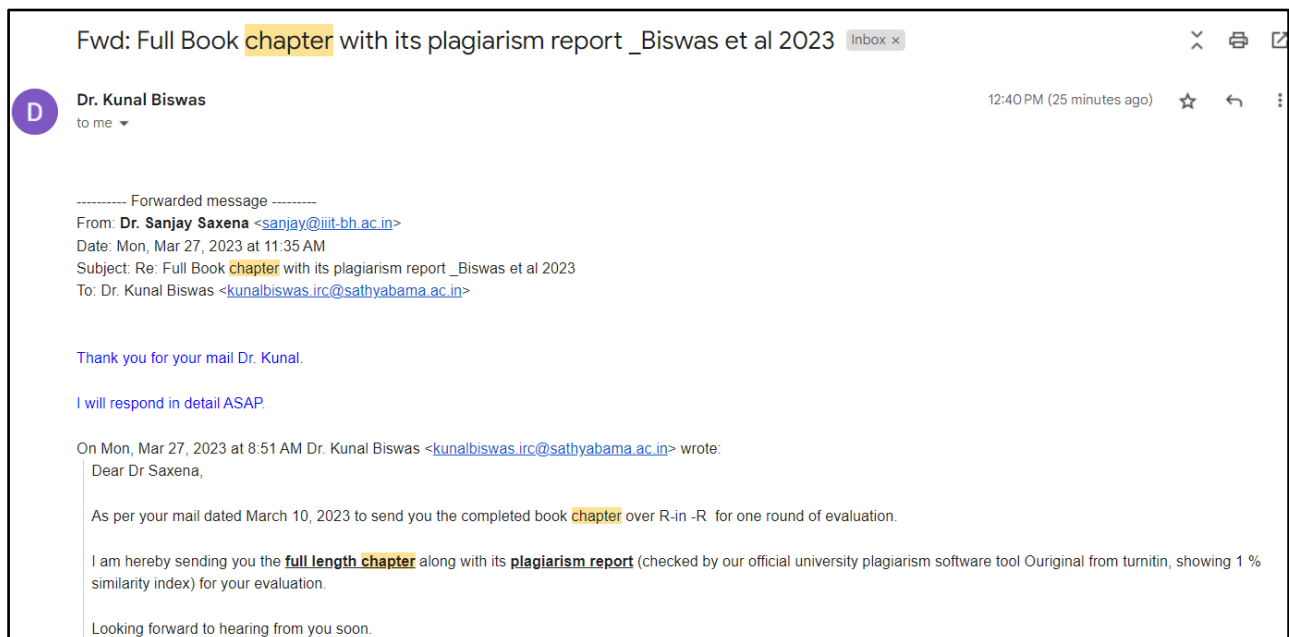
2. Integration of Technology and Nanoscience in Precision agriculture and farming (IGI Global) (Book chapter accepted)



3. Radiomics and Radiogenomics in Neuro-Oncology: An Artificial Intelligence Paradigm (Elsevier) (Abstract accepted, Full chapter submitted)



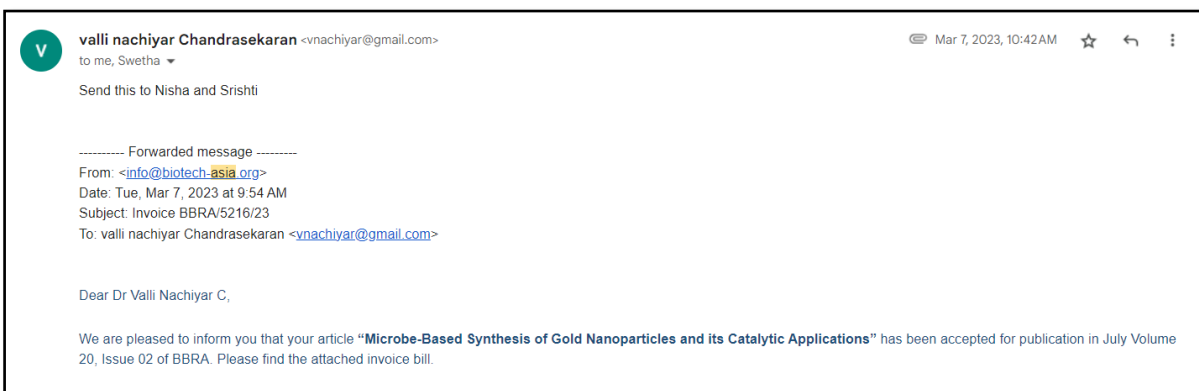
4. AI for healthcare applications solutions to healthcare services using AI detection and diagnosis of different diseases using AI(Abstract accepted, Full chapter submitted)



5. Microbe-Based Synthesis of Gold Nanoparticles and its Catalytic Applications: Book Chapter (Manuscript No-BBRA/5216)

Accepted in Biosciences Biotechnology Research Asia Journal
SCOPUS, Clarivate Analytics, EBSCO, ROAD and ProQuest indexed.

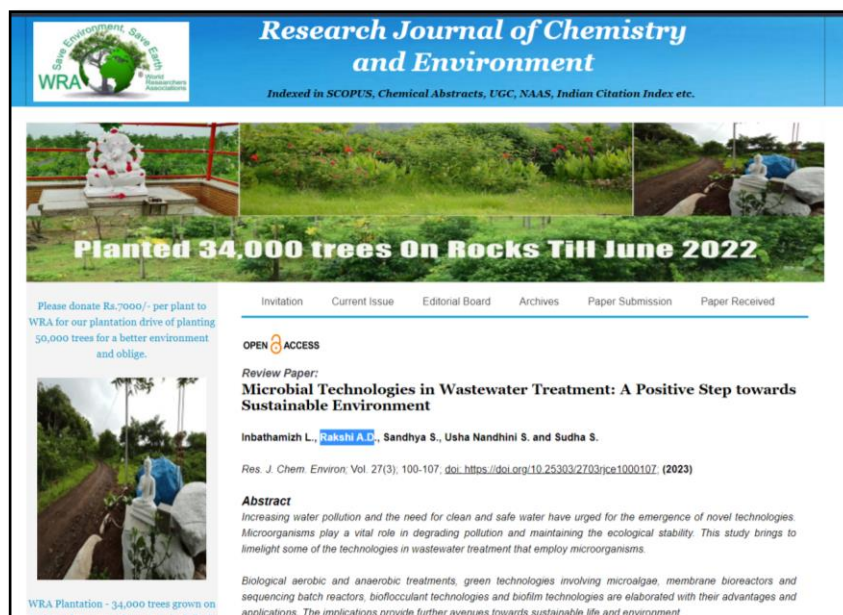
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