FABRICATION AND ASSESSMENT OF REDUCED GRAPHENE OXIDE AND SILVER NANOCOMPOSITES USING *Clinacanthus nutans* (Burm.f.) Lindau FOR BIOMEDICAL APPLICATIONS

By

DHARSHINI A/P PARMAL @ PERUMAL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair : Che Azurahanim Che Abdullah, PhD
Faculty : Science

Graphene derivatives have garnered substantial interest due to their remarkable properties. The incorporation of graphene derivatives with metal nanoparticles forms nanocomposite that enhances their properties and potential applications in the field of medicine. Silver nanoparticles (AgNPs) are extensively utilised due to their excellent physical and biological properties. During the synthesis process, uncontrolled growth of silver ion can lead to agglomeration. This makes them to lose their desirable properties. Graphene derivative serves as a good support to stabilize AgNPs. The fabrication of nanocomposite employs a green synthesis compared to conventional physical and chemical methods. The green synthesis utilising natural and renewable resources is cost-effective, environmentally friendly, and non-toxic. In the present study, one pot synthesis of reduced graphene oxide decorated silver (rGO-Ag) nanocomposite was successfully prepared utilizing the extract of medicinal plant, *Clinacanthus nutans*. The extract makes it a potent reducing agent for the reduction of graphene oxide (GO) and silver nitrate solution as a precursor to produce nanocomposite. The synthesis of rGO-Ag nanocomposite was conducted using the selected conditions: 100 °C (temperature), 50 % (leaf extract concentration), and 6h (time). The properties of rGO-Ag nanocomposite were characterized by ultraviolet-visible spectroscopy (UV-Vis), x-ray diffraction spectroscopy (XRD), Fourier transform infrared spectroscopy (FTIR), raman spectroscopy, field emission scanning electron microscopy (FESEM), and energy dispersive X-ray spectroscopy (EDS). The absorbance peak observed at 425 nm and 263 nm indicates the formation of rGO-Ag nanocomposite. The XRD and FTIR pattern showed that the rGO-Ag nanocomposite matched the pattern of AgNPs. The raman analysis showed a D to G peak intensity ratio of 0.56. FESEM image revealed the spherical shaped AgNPs of average size 48 ± 11 nm deposited on the surface of rGO.

The EDS analysis revealed the weight ratio of Ag (84.3 %) and C (9.1 %). The antibacterial activity of the as-synthesized sample against *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S. aureus*) bacterial strain were studied. GO, leaf extract, and rGO did not show inhibitory activity whereas AgNPs demonstrated the best antibacterial activity against *E. coli* compared to *S. aureus*. The rGO-Ag nanocomposite showed excellent antibacterial activity against both *E. coli* (11.86 ± 0.29 mm) and *S. aureus* (11.99 ± 0.26 mm) strains. The as-synthesized sample was also assessed for their potential as anticancer agent against human lung cancer cell (A549) and human epithelial colorectal cancer cell (Caco2) using XTT assay. GO and rGO showed low cytotoxicity against A549 cells while no cytotoxicity against Caco2 cells. AgNPs displayed dose-dependent cytotoxicity against both cells. At the highest concentration of 25 µg/mL, the cell viability of rGO-Ag nanocomposite towards A549 cells was 80 % (IC_{50} value 3.509 µg/mL) and Caco2 cells was 79 % (IC_{50} value 2.578 µg/mL), respectively. The ecotoxicity studies revealed that at low concentration (0.001 mg/mL) rGO-Ag nanocomposite exhibit more than 50 % hatching rate of *Artemia salina* cysts. In summary, this work provides the promising green synthesis by utilizing non-toxic and environmentally friendly reducing agent to produce rGO-Ag nanocomposite for biomedical applications.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBINAAN DAN PENILAIAN KOMPOSIT GRAFIN OKSIDA TERTURUN DAN PERAK MENGGUNAKAN *Clinacanthus nutans* (Burm.f.) Lindau UNTUK APLIKASI BIOPERUBATAN

Oleh

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Grafin terbitan menerima minat yang kukuh disebabkan sifat yang menakjubkan. Pemerbadanan grafin derivatif dengan zarah nano logam untuk membentuk komposit nano mempertingkatkan sifat dan mempunyai potensi aplikasi dalam bidang perubatan. Zarah nano perak digunakan secara meluas disebabkan sifat fizikal dan biologi yang cemerlang. Semasa proses sintesis, pertumbuhan ion perak menyebabkan penggumpalan. Ini menyebabkan mereka kehilangan sifat yang diingini. Grafin terbitan memberi sokongan baik untuk penstabilan AgNPs. Penghasilan komposit nano menggunakan sintesis hijau berbanding dengan kaedah konvensional fizikal dan kimia. Sintesis hijau menggunakan bahan semula jadi dan boleh diperbaharui adalah kos efektif, mesra alam, dan tidak toksik. Dalam kajian ini, satu pot sintesis melibatkan komposit nano grafin oksida terturun berhias perak (rGO-Ag) berjaya disediakan menggunakan ekstrak tumbuhan ubatan, *Clinacnathus nutans*. Ekstak menjadikan ia agen pengurangan mujarab untuk pengurangan cecair grafin oksida (GO) dan perak nitrat sebagai pendahulu untuk menghasilkan komposit nano. Sintesis komposit nano rGO-Ag dijalankan menggunakan kondisi yang dipilih: 100 °C (suhu), 50 % (kepekatan ekstrak daun), dan 6 h (tempoh). Sifat komposit nano rGO-Ag dicirikan menggunakan spektroskopi ultralembayung boleh nampak (UV-Vis), spektroskopi pembelauan sinar-x (XRD), spektroskopi inframerah transformasi Fourier (FTIR), spektroskopi raman, mikroskop imbasan elektron pancaran medan (FESEM) dan spektroskopi penyerakan tenaga sinar-x (EDS). Puncak penyerapan yang dilihat pada 425 nm dan 263 nm menunjukkan pembentukkan komposit nano rGO-Ag. Corak XRD dan FTIR menunjukkan komposit nano rGO-Ag sepadan dengan corak AgNPs. Analisis raman menujukkan nisbah intensi puncak D kepada G adalah 0.56. Imej FESEM mendedahkan AgNPs berbentuk sfera

yang mempunyai saiz purata 48 ± 11 nm mendap di atas permukaan rGO. Analisis EDS mendedahkan nisbah berat Ag (84.3 %) dan C (9.1 %). Aktiviti anti-bakteria oleh sampel sebagai-sintesis terhadap *Escherichia coli* (*E.coli*) dan *Staphylococcus aureus* (*S. aureus*) strain bakteria telah dikaji. GO, ekstrak daun, dan rGO tidak menunjukkan sebarang aktiviti penhalang manakala AgNPs mendemonstrasi aktiviti anti-bakteria yang terbaik terhadap *E. coli* berbanding dengan *S. aureus*. Komposit nano rGO-Ag menunjukkan anti-bakteria yang cemerlang terhadap kedua-dua *E. coli* (11.86 ± 0.29 mm) dan *S. aureus* (11.99 ± 0.26 mm) strain. Sampel sebagai-sintesis dinilai untuk berpotensi sebagai agen anti-kanser terhadap sel kanser paru-paru manusia (A549) dan sel kanser kolorektal epithelium manusia (Caco2) menggunakan asai XTT. GO and rGO menunjukkan kesitotoksikan rendah terhadap sel A549 sementara tiada kesitotoksikan terhadap sel Caco2. AgNPs mempaparkan ketoksikan bergantung kepada dos terhadap kedua-dua sel. Pada kepekatan tertinggi 25 µg/mL, daya maju sel komposit nano rGO-Ag ke arah sel A549 adalah 80 % (IC_{50} value 3.509 µg/mL) dan sel Caco2 adalah 79 % (IC_{50} value 2.578 µg/mL), masing-masing. Kajian ekotoksisitas menunjukkan pada kepekatan rendah (0.001 mg/mL) komposit nano rGO-Ag mempamerkan lebih daripada 50 % kadar penetasan sista *Artemia salina*. Secara ringkasnya, kajian ini memberikan harapan untuk sintesis hijau menggunakan agen pengurangan yang tidak toksik dan mesra alam untuk menghasilkan komposit nano rGO-Ag untuk aplikasi bioperubatan.

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LIST OF ABBREVIATIONS

GO	Graphene oxide
rGO	Reduced graphene oxide
AgNPs	Silver nanoparticles
rGO-Ag	Reduced graphene oxide decorated silver
Ag-GO	Silver-graphene oxide
PGE-HRG-Ag	Silver doped highly reduced graphene oxide synthesised using *Pulicaria glutinosa* extract
C. nutans	*Clinacanthus nutans*
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
CCK-8	Cell Counting Kit-8
WST-8	2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium
A549	Human lung cancer cells
Caco2	Human epithelial colorectal cancer cells
MCF-7	Human breast cancer cells
HepG2	Human liver cancer cells
DU-145	Human prostate cancer cells
Hela	Human cervical cancer cells
A2780	Ovarian cancer cells
A. salina	*Artemia salina*

Ag	Silver
UV-Vis	Ultraviolet-visible spectroscopy
XRD	X-ray diffraction spectroscopy
FTIR	Fourier transform infrared spectroscopy
FESEM	Field emission scanning electron microscopy
EDS	Energy dispersive X-ray spectroscopy
CHNS/O	Carbon, Hydrogen, Nitrogen, Sulphur/ Oxygen
$AgNO_3$	Silver nitrate
Au	Gold
Pt	Platinum
Cu	Copper
Pd	Palladium
Ni	Nickel
B. cereus	*Bacillus cereus*
S. typhimurium	*Salmonella typhimurium*
P. aeruginosa	*Pseudomonas aeruginosa*
S. mutans	*Streptococcus mutans*
E. cloacae	*Enterobacter cloacae*
MIC	Minimum inhibitory concentration
H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid

$KClO_3$	Potassium chlorate
HNO_3	Nitric acid
$NaNO_3$	Sodium nitrate
$KMnO_4$	Potassium permanganate
ATCC	American Type Culture Collection
LB	Luria Bertani
DMEM	Dulbecco's Modified Eagle Medium
RPMI	Roswell Park Memorial Institute
PMS	Phenazine Methosulfate
FBS	Fetal bovine serum
v/v	Volume/volume
Kα	K alpha emission
C	Carbon
H	Hydrogen
N	Nitrogen
S	Sulphur
O	Oxygen
NaCl	Sodium chloride
Cl	Chlorine
Ca	Calcium
Mg	Magnesium
Si	Silicon
SD	Standard deviation

CHAPTER 1

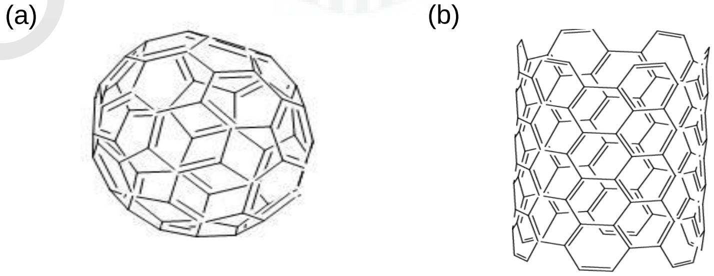
INTRODUCTION

1.1 Research background

Nanotechnology is a field of study that encompasses several areas of material design and is currently developing at the nanoscale level. Applications for materials are numerous and diverse, including electronics, nutrition, textiles, cosmetics, medicine, energy production, and so on (Nasar *et al*., 2019). This application is made possible by the creation of nanoparticles. Nanoparticles are a diverse class of materials with overall dimensions less than 100 nm on the nanoscale (Khan *et al*., 2019). Nanoparticles are remarkably powerful in terms of their physical strength, chemical reactivity, aesthetic effects, electrical conductivity, and magnetism due to their small dimension (Adebayo-Tayo *et al*., 2019).

Nanoparticles are divided into four categories: zero-dimensional (0D), one-dimensional (1D), two- dimensional (2D), and three-dimensional (3D) (Mohamed, 2017). Nanoparticles are classified into several types based on their shape, dimension, and chemical properties. Semiconductor, ceramic, carbon,polymeric, lipid-based, and metal nanoparticles, are all included (I. Khan *et al*., 2019). Carbon nanoparticles are increasingly being used in biological applications such as tissue engineering, cancer therapy, drug delivery, diagnosis, and biosensors (Maiti *et al*., 2019). Carbon being light weight is regarded as a flexible material component because it can adopt a variety of structures with different bonding possibilities, resulting in carbon allotropes with varying properties. All allotropic carbonic materials are primarily composed of carbon atoms as depicts in Figure 1.1. Graphene and its derivatives, graphene oxide (GO) and reduced graphene oxide (rGO), are two ofthe most well-known forms of carbon (Tarcan *et al*., 2020).

(a) (b)



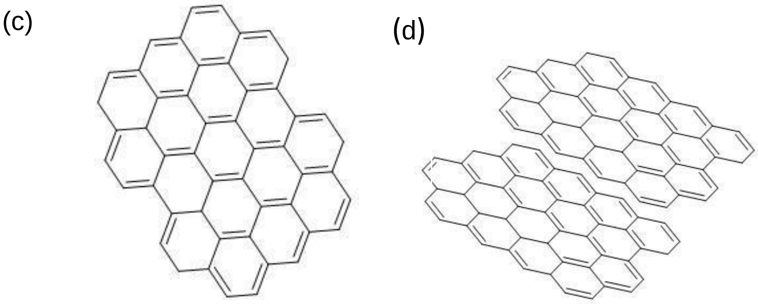


Figure 1.1: Graphic image of (a) a fullerene (0D), (b) a single-walled carbon nanotube (1D), (c) a graphene (2D), and (d) a stacked graphite (3D)

Graphene, a one-of-a-kind two-dimensional sp^2 hybridized substance with a honeycomb-like structure, has piqued the interest of researchers due to its mechanical, thermal, chemical, and physical properties, particularly its large surface area (Dominic *et al*., 2022). Graphene can be synthesized using chemical vapor depositions, pyrolysis, laser ablation, mechanical exfoliation, and liquid phase exfoliation (Goyat *et al*., 2022). The hydrophobic characteristic of graphene prevents its usage in the biomedical field (Syama & Mohanan, 2019). A range of methods for producing and utilizing graphene-based materials have recently been developed. GO and rGO,the two most prominent graphene derivatives, have been studied for a myriad of medical applications ranging from therapeutic drug delivery to diagnostic imaging. This is because of theirdistinct physiochemical properties, renewability, readily available, and low-cost basic materials (Joshi *et al*., 2020).

GO can be made by oxidizing graphite to graphite oxide and then exfoliating it (Jiříčková *et al*., 2022). GO is defined by oxygen-containing functional groups, a substantial surface area, and strong dispersion properties (Yang *et al*., 2021). The oxygen functional groups broadens the interlayer gaps that enables them to be functionalized with by small molecules or polymers via covalent or non-covalent interactions (Yu *et al*., 2020). In addition to its widespread use in electronics, optics, and wastewatertreatment, GO has evolved into a promising and workable material for biomedical applications (Yang *et al*., 2021). GO can be synthesized most commonly utilizing the Brodie, Staudenmaier, or Hummer's method (Pang et al., 2018). These methods use strong oxidizing agents leading to significant amounts of defects in its crystalline network. Hence, these makes the properties of GO inferior to graphene (Ghulam et al., 2022).

To resolve this issue, it is possible to perform reduction treatments on GO to produce rGO using reductants (Figure 1.2). Reduction treatments such as thermal, chemical and electrochemical was utilized to remove the oxygen functional groups (Smith *et al*., 2019). rGO synthesis is preferred due to its facile fabrication, improved properties, and ability to be incorporated into numerous applications

(Priyadarsini *et al*., 2018). The effectiveness of reducing agent determines the degree of oxygen elimination. The deoxygenation, however, is not complete, and the residual oxygen functions encourage rGO dispersion, functionalization, and interaction with polymers (Barra *et al*., 2022). rGO serves as a template to support metal nanoparticle nucleation, growth, and attachments (Ng et al., 2019).

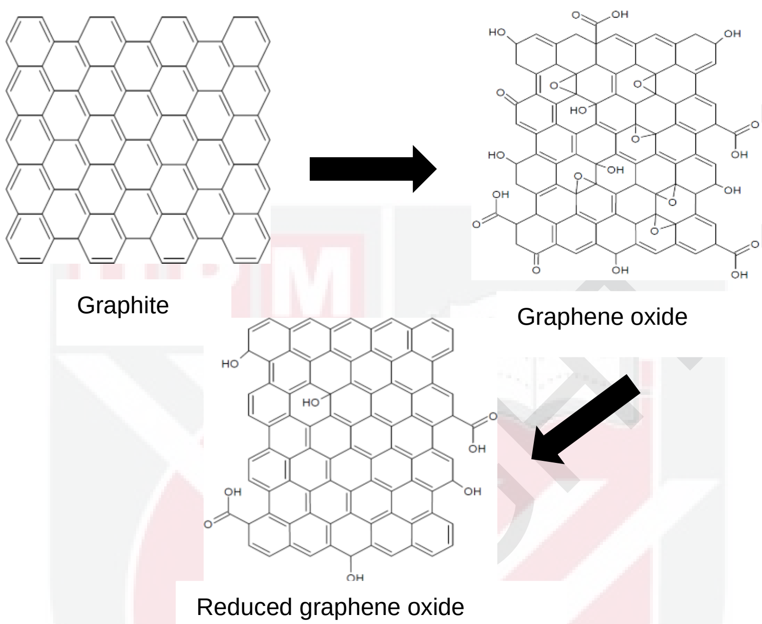


Figure 1.2: GO and rGO preparation is shown schematically

Metal nanoparticles are becoming popular in the scientific community due to their intriguing potential in the domains of material science, pharmacology, and biology (Thiyagarajulu & Arumugam, 2020). Silver nanoparticles (AgNPs), one of the noble metal nanoparticles, has gained importance recently due to their remarkable properties (Erci *et al*., 2018). Several studies have shown that AgNPs have antimicrobial, antioxidant, antiviral, anticancer, antibacterial, antifungal, and antiplatelet properties (Gupta *et al*., 2020; Neupane *et al*., 2022; Shahi *et al*., 2018). The biological activity of silver (Ag) has been discovered to be correlated with the size of the Ag particle, the smaller the particle size, the more effective the antibacterial activity. However, as the size of the Ag particles decreases, surface energy rises, and the particles tend to agglomerate, stabilising agents such as surfactants or support materials are typically added (H. Wang et al., 2020).

Nanocomposites incorporating noble metal nanoparticles and carbon nanomaterials would significantly enhance biological applications due to their distinctive physicochemical features, high surface area, and strong inhibitory activity (Kavinkumar *et al*., 2017). The development of rGO and metal/metal oxide-based nanocomposite are currently receiving a lot of interest from researchers (Abazari *et al*., 2022; Richtera *et al*., 2015). Therefore, combining the qualities of rGO and Ag in nanoscale metal-rGO composites would be interesting for the creation of materials with antibacterial and antifungal studies as well as anticancer studies (Gurunathan et al., 2015). Figure 1.3 depicts some of the applications of reduced graphene oxide-silver (rGO- Ag) nanocomposite as reported in previous literature (Ali *et al*., 2022; Chen *et al*., 2016; Darabdhara *et al*., 2019; Devi *et al*., 2022; Mamo & Zeleke, 2022; Shafi *et al*., 2022; C. Wang *et al*., 2017).

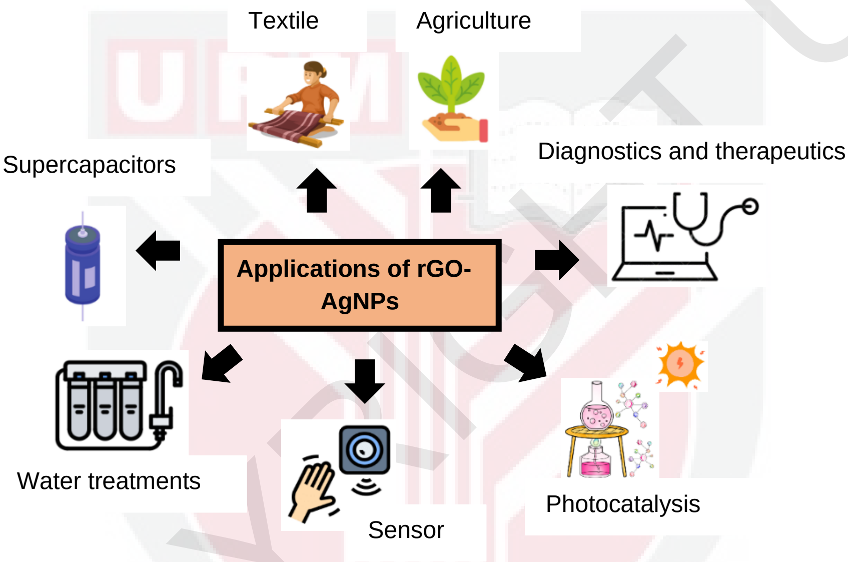


Figure 1.3: Applications of rGO-AgNPs

Metal nanoparticles combining graphene and other allotropes are created using a variety of synthesis technique such as chemical vapor deposition, gamma irradiation, chemical reduction, and sonochemical method. The most popular of these techniques is the chemical reduction of Ag ion and GO into rGO-Ag nanocomposite in the presence of reducing agents since it is scalable and repeatable (Devi et al., 2022). However, the reducing agents pose a threat to the environment and human health due to their explosive and toxic nature (Kulshrestha *et al*., 2017). In addition, some harsh chemicals deposits on the surface of the nanocomposite reduces their performances and making it undesirable to be utilised in the cosmetics, pharmaceutical, and medical industries (Gurunathan *et al*., 2015; Veisi *et al*., 2019).

These factors are motivating researchers to prioritise environmentally friendly and sustainable reduction methodologies for plant extract synthesis (Bandeira *et al*., 2020). A "green approach" is an uncomplicated, economical procedure that does not require high temperatures or abrasive reduction agents and involves parts and methods that lessen the usage and manufacture of hazardous compounds (Hareesh *et al*., 2016). The diverse climate of Malaysia has resulted in a diverse range of natural habitats and vegetation zones.

Throughout history, people have used natural resources such as medicinal plants as sources of remedies. A wide range of bioactive substances found in medicinal plants have been used for a broad array of purposes, including in the prevention, diagnosis, improvement, and treatment of a wide range of human physical illnesses (Haida & Hakiman, 2019). There are currently over 350 000 higher plant species in the wild, with approximately 80 000 species having pharmacological potential (Chia *et al*., 2022).

The current study sought to investigate the reduction performance of rGO, AgNPs, and rGO-Ag nanocomposite using *Clinacanthus nutans* leaf extract (*C. nutans*). The as-synthesized samples were characterized using various spectroscopy and microscopy techniques. The antibacterial potential of as-produced nanoparticles and nanocomposite was investigated using microbial species of *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S. aureus*). The XTT assay was used in an *in vitro* study against human lung (A549)and human epithelial colorectal (Caco2) adenocarcinoma cell lines. Our team also conducted a toxicity analysis of the biosynthesized nanoparticles and nanocomposites against *Artemia salina* (*A. salina*) brine shrimp cysts. We proposed that these biosafe extracts act as a reducing and stabilising agent, as well as a database for future research.

1.2 Problem statement

A two-dimensional carbon material, graphene has attracted great interest in recent years due to its unique properties including large surface area, excellent mechanical, electrical, electron mobility, and optical properties (Abbas et al., 2022). Graphene and its derivatives including GO and rGO have been widely applied in biomedical fields due to its superior properties (Syama & Mohanan, 2019; Y. Wang et al., 2022). rGO have reportedly been used as antimicrobials, compounds that promote wound healing, and anticancer agents (El-Zahed et al., 2021). Research has shown that the green tea extract used in rGO as a reducing agent has outstanding anticancer, antioxidant, and antibacterial potential (Vatandost *et al*., 2020). In comparison to GO, rGO produced from *Chenopodium album* leaf extract demonstrated higher antibacterial and antibiofilm activity as well as powerful anticancer agent (Umar et al., 2020).

Metal nanoparticles, especially Ag, have exhibited remarkable optical, mechanical, and thermal properties as well as biological properties (Zhang *et al*., 2016). AgNPs have been tested in the field of biomedicine because of their high cytotoxicity and low resistance. AgNPs produced from *Syzgium cumini* fruit extract had strong antioxidant, anti-inflammatory, and antibacterial activity (Chakravarty et al., 2022). The synthesis of AgNPs utilizing the leaf extract of the Copperpod plant (*Peltophorum pterocarpum*) resulted in cytotoxicity against malignant cells, with IC_{50} values for hepatocellular carcinoma (HepG2), breast cancer cells (MCF-7), and lung cancer (A549) being 69, 62, and 53 µg/mL, respectively (Pannerselvam et al., 2021). Despite having remarkable qualities, AgNPs are prone to aggregate due to their high surface energy (Barjola et al., 2022). As a result, these lessen their biological activity (Krishnaraj et al., 2022).

The incorporation of metal nanoparticles on graphene-based materials to form nanocomposite has been reported to improve the characteristics for variety of applications. GO is a great carrier for metal nanoparticles because it has a lot of surface area and oxygen functional groups (H. Wang et al., 2020). High drug loading capacity for acetylsalicylic acid was demonstrated by rGO-Ag nanocomposite synthesized using sodium borohydride (Handayani et al., 2022). Krishnaraj and co-workers successfully fabricated rGO-Ag nanocomposite using *Angelica keiskei* leaf extract. According to their research, rGO-Ag nanocomposite exhibits strong antibacterial activity, has minimal cytotoxicity, and considerably lessen the toxic effects on zebrafish embryos, demonstrating their biocompatibility, (Krishnaraj et al., 2022).

The fabrication of nanocomposite has been reported by different preparation approaches, which includes chemical, physical and biological methods (Ahamed *et al*., 2021). The physical methods are expensive, low production rate, and high energy consumption are the major limitations. Conversely, chemical methods use reducing agents such as hydrazine, formaldehyde, sodium borohydride, potassium bitartrate during the synthesis. Chemical hydrazine is a highly toxic and potentially explosive, therefore, its use should be avoided in the large-scale implementation (Fernandez-Merino *et al*., 2010). The sodium borohydride is corrosive, flammable, and toxic (Silva *et al*., 2014). Although the chemical method are economical for large-scale production, the use of the toxic chemicals and production of harmful by-products cause environmental damage (Dikshit et al., 2021) as well limits the usage in biomedical field (Ahamed et al., 2021). The harsh chemicals absorb on the surfaces of the nanoparticles improving the issues of toxicity (Hemmati et al., 2018). In addition, the aquatic life has been experiencing issues pertaining to ecological toxicity due to the uncontrolled use and improper disposal of products industrial waste and sewage sludge (Ahmed et al., 2022).

To overcome the problem related to physical and chemical methods researchers have shown interest in an environmentally friendly, cost-effective, and non-toxic green method for synthesizing nanocomposites. Hence, the biological method involves the usage of plant extracts and microorganisms have become an alternative option (Sivanesan et al., 2021). Plant extract is preferred because it is a more impromptu technique, does not require specific care such as isolation, culture, or culture maintenance, and effortless to scale up (Oves et al., 2022; Sharma et al., 2022). rGO-Ag nanocomposite synthesized using orange peel extract demonstrated two-fold killing potential against MCF-7 and A549 cancer cell lines compared to pure AgNPs (Ahamed et al., 2021). *Kigelia Africana* stem extract was utilized for the synthesis of rGO-Ag nanocomposite showed superior antioxidant and antibacterial activity (Kurmarayuni et al., 2022).

Belonging to the Acanthaceae family, *Clinacanthus nutans* (*C. nutans*) is a shrub native to tropical Asian countries, mainly Malaysia, Thailand, and Indonesia (Hashim *et al*., 2019). The pharmacological studies on *C. nutans* revealed that this plant exhibited anti-inflammatory, antioxidant, anti-diabetic, anti-rheumatism, and antiviral activities (Chithra *et al*., 2016; Hashim *et al*., 2019). Studies have also shown that *C. nutans* contains bioactive compounds such as lipids, chlorophyll derivatives, glucosides, and benzenoids as well as steroids, terpenoids, phenolics. The bioactivities of these substances are recognized to be advantageous to human health (Lim et al., 2022). Despite the excellent bioactivities, the human and environmental toxicity of the nanocomposites is rarely reported.

Herein, in the current study, it is the first report where we have fabricated reduced graphene oxide decorated silver (rGO-Ag) nanocomposite using leaf extract of *C. nutans*. The synthesis, characterisation as well as the bioactivity investigations have been reported for their performance in biomedical applications. The toxic effects were examined using *Artemia salina* cysts as the model organism.

1.3 Objectives

This thesis describes the development of rGO-Ag nanocomposite for potential biomedical applications.This study is intended to accomplish the following goals:

I. To synthesize rGO, Ag, and rGO-Ag nanocomposite via green synthesis using *C. nutans* leaf extract.

II. To investigate their structural and optical properties using Ultraviolet-visible spectroscopy (UV-vis), X-ray diffraction spectroscopy (XRD), Fourier transform infrared spectroscopy(FTIR), Raman spectroscopy, Field emission scanning electron microscopy (FESEM), and energy dispersive X-ray spectroscopy (EDS).

III. To evaluate the samples antibacterial effect against gram-negative (*E. coli*) and gram-positive(*S. aureus*) bacterial strains using disc diffusion assay.

IV. To assess the *in vitro* cytotoxicity of the synthesized sample towards A549 and Caco2 adenocarcinoma cell lines using XTT assay.

V. To evaluate the toxicity of the synthesized samples using brine shrimp (*A. salina*) hatching assay.

1.4 Scope of study

This study used GO and silver nitrate ($AgNO_3$) solution as a precursor to synthesis a rGO-Ag nanocomposite. Improved Hummer's method was used to synthesis GO. The synthesised GO was then reduced using parameters such as temperature, leaf extract concentration, and time to form rGO. Furthermore, the same parameter was used to optimise AgNPs synthesis. The optimised parameter was then used to synthesis rGO-Ag nanocomposite.

The physicochemical properties of the synthesized samples, including GO, rGO, AgNPs, and rGO-Ag nanocomposite, were investigated utilising a variety of characterization techniques. UV-Vis spectroscopy is used to measure the surface plasmon resonance in order to verify the creation of nanoparticles. XRD was used to investigate the crystallinity of nanoparticles. The identification of functional groups in nanoparticles was accomplished through the use of FTIR. The chemical structure of the material was examined using Raman spectroscopy. FESEM was used to determinemorphology. EDS was used to study the elemental content of the sample.

The antibacterial effect of the as-prepared samples was tested using a disc diffusion assay against gram-negative and gram-positive bacteria. An *in vitro* cytotoxicity test on the as-synthesized samples was performed against A549 and Caco2 cancer cell using XTT assay with varying sample concentrations. The samples ecotoxicity was determined using the brine shrimp hatching assay.

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