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RESEARCH PAPER

# Effects of Amination on Graphene Oxide Properties: Study of Antioxidant and Antimicrobial Activities

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**ABSTRACT**

This research investigated the synthesis and applications of various functionalized graphene oxide (GO) derivatives with amine groups. Graphene oxide was synthesized using the Hummers' method, yielding a black powder with a 68% yield. Its properties were studied using FT-IR spectroscopy, 1H-NMR, FESEM, and XRD analysis, showing consistency with previous literature. Subsequently, several GO derivatives were prepared by incorporating amine compounds, such as 3-aminophenol (GO-1), 4-aminouracil (GO-2), 2-aminobenzoic acid (GO-3), naphthylamine (GO-4), and o-anisidine (GO-5). Spectroscopic and XRD analyses of these derivatives revealed changes in structural and compositional properties, indicating successful functionalization. The antimicrobial and antioxidant activities of the functionalized GO derivatives were evaluated. Antibacterial activity tests showed that GO-1 was the most effective against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli, surpassing conventional antibiotics such as ampicillin. For antifungal activity, comparative data with fluconazole were not available, but previous studies suggest that functionalized graphene oxide may possess antifungal properties. In the antioxidant activity test using the DPPH assay, the derivatives displayed significant variations in their ability to scavenge free radicals. Among these derivatives, GO-1 demonstrated the highest antioxidant activity with a scavenging rate of up to 56.3% at a concentration of 75 µg/mL, and an IC50 value of 25.4 µg/mL. These results support the effectiveness of functional modifications on graphene oxide in enhancing its antioxidant properties. Amine-modified graphene oxide has improved properties in anti-oxidant and anti-microbial applications, that is interesting for environment and medical applications.

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## INTRODUCTION

Graphene is a nanomaterial of connected carbon atoms that continues in growing sheets, one atom thick; carbon only reserves the correct atoms number in connections with other carbons to form a layer of atoms. Topological Chronicle: On the horizon, the Soviet flag rises. The year is 2004, and Andre Geim and Konstantin Novoselov have just used sticky tape to isolate graphene[1]. Can you conceive of a more groundbreaking

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revolution? No you could not, and neither could the Nobel Prize in Physics, who eagerly snatched it up in 2010. A comprehensive examination of the physical and chemical characteristics of graphene has led to its recognition to be one of the preeminent contemporary material in the classical fied of materials science [2]. Such is its exceptional thermal and electrical conductivity, mechanical robustness, and highly flexible nature, this material is a prime candidate for numerous and varying technological applications [3]. GO was produced by oxidation of graphite with strong oxidizing agents. As the process went on, oxygen functional groups that included hydroxyl, carboxyl and lactone were being introduced on the surface of the graphene. The functional groups improve the reactivity of graphene oxide with other materials, and improve the solubility in different solvents and other mediums, making graphene oxide a more useful species to work with in different applications [4]. Graphene oxide is an incredibly useful material with a wide variety of applications due to its versatility in being able to be chemically and physically altered in ways that will have an impact on each field. Graphene oxide derivatives are compounds in which the chemical structure of GO has been modified to enable the enhancement and/or inclusion of new functions. The modifications of Graphene oxide, which are basically the incorporation of different chemical groups including: amine groups, organic acids, and various nanomaterials to improve the properties of Graphene oxide[5]. In pharmaceutical fields the implied amine groups can enhance not only antibacterial activity but also drug delivery efficiency. Graphene oxide and its derivatives are vital in several critical domains. The use of graphene oxide (GO) and its derivatives in the field of advanced electronics is an important and widely studied topic in materials chemistry. Most significantly, GO is a basic building block for the development of high-performance devices such as transistors, supercapacitors, and sensors. The use of Graphen Oxide based materials results in a dramatic increase in electrical conductivity, which allows these materials to be used in the creation of more efficient electronic circuits and more advanced flexible electronic devices[6]. In addition to this, in medicine and medical imaging, as well as biotechnology, graphene oxide derivatives have shown potential in applications such as drug delivery and gene therapy. Not only are these materials precise in their ability to interact with cells and tissues, but they pristinely up the ante of treatments and offer new divisions of diagnosis and therapy. Graphene oxide is used in creating new energy storage technology including supercapacitors, fuel cells and devices for the conversion of solar energy in the energy sector [7]. The superior electrical conductivity and thermal conductivity of graphene oxide contribute significantly to energy efficiency and promoting the lifespan of the batteries. Environmental applications are another large opportunity. Graphene oxide derivatives are used for water treatment, purification of air, and pollution control, in this context as well. These substances can break down dangerous molecules in air and water and can then remove them in the manner in which they do so, air and water pollutants can be ingested or adhered to[8]. In a notable research endeavor, an inquiry had been made into the planning of graphene oxide and its auxiliaries and additionally its portrayal. Through this study, we hope to attain an understanding of the chemical interactions between graphene oxide and a range of other materials and thereby how these interactions affect the physical and chemical properties of graphene oxide[9] . According to the study, The use of graphene oxide as a carrier to ameliorate the mechanical and chemical properties of various materials, boosts its applicability to the technology based industrial sector. Subsequent to Collation has occurred to appraise graphene oxide's cursory applications these are electronics and composite applications . The examination actually looked at the benefits of using graphene oxide in electronics as well as its high electrical conductivity. Graphene oxide would helpful in improving the performance of composite materials. Graphene oxide has been identified as holding the potential to radically improve the ability to manufacture superior electronic devices like transistors and su Conducting a thorough/ holistic/exhaustive systematic review over diverse and various/modifications/alterations of Graphene oxide properties for Biomedical and Environmental Applications[10]. The purpose of the study was based on the graphene oxide derivatives and the possibility of using them as antimicrobial agents, their therapeutic materials, and chemical modification to improve their effectiveness. Examining the reversible Co(II) interaction with graphene oxide (GO) enables

the research of its possible applications in water treatment and air purification processes[11,12]. An examination into the basic properties of graphene and explanation about the electron transport through graphene giving some new prospective about the future implementation of electronic devices. The research underscored that graphene has extraordinary physical and electrical characteristics [13]. This material could be the future of the giant leaps in the electronics [14]. A thorough exploration of applications of graphene oxide through a various environmental media within water treatment and pollutant remediation was previously baffled. The aim of the project was to spin up the effectiveness of water desalination procedure by using graphene oxide in collaboration with its main derivatives. Graphene oxide and its derivatives essentially have high capacity of absorbing harmful molecules [15-17]. Delving deep into the applications of graphene oxide in the wide array of fields medicine has to offer, summaries are given on how it behold a hopeful innovation in the world of drug delivery, gene therapy and even medical imaging. The examination accentuated the vast capability of graphene oxide in revolutionizing the aptitude of pharmaceutical treatments and diagnostic procedure [18]. The present review systematically summarizes the recent progress in the synthesis of graphene oxide (GO) and GO derivatives, and emphasizes, from both the viewpoint of material chemistry and functional materials application, the newly developed techniques are effective on improving the quality and the properties of GO and its derivatives [19]. Also researched were the new industrial uses of Graphene Oxide in the areas of energy, electronic, and environmental science [20]. Used for drug delivery system, graphene oxide is able to help with increasing the drug's health giving abilities. The large area of graphene allows many drugs to fit onto them, making graphene oxide the perfect delivery system for targeted drug delivery to specific cells [21]. In addition, since graphene oxide can be functionalized with organic molecules, this nanomaterial can be utilized as a vector for targeted drug delivery, in which many therapeutics can be included and driven to the precise cancerous cells or affected tissues by functionalizing on the unique π-π stacking behavior between the organic molecules and the carbon sources. By having such specific delivery method, the collateral damage due to the non-specificity of the majority of today's drugs can be limited to minimum [22].

Graphene oxide has the potential to revolutionise the development of more sophisticated diagnostic tools in the field of biotechnology such as biosensors, in which the addition of graphene oxide can result in much higher sensitivity in the detection of a range of biological molecules and drugs. In addition, it is used within gene delivery systems. This helps genes to be inserted into cells accurately, therefore improving the effectiveness of gene therapies. When graphene oxide is used with antibiotic chemicals, it gets the immune system working more effectively. By being able to augment reactions that occur chemically the effectiveness of antibiotics in eradicating bacterial infections is improved. In addition, graphene oxide has use in the development of new antibiotics as a universal panel in medicine [23]. Its distinctively unusual and unique features could potentially help the overall development and coming together of certain very effective compounds that could target, and attack many bacteria or certain other certain bacterial strains and certain microbes. The antimicrobial properties seen in graphene oxide are excellent in suppressing the growth rate of a broad spectrum of both bacteria and fungi. This phenomenon is attributed to its low pH which make these microbes disturbed in their metabolic activities which also disturbs in their Biochemical activities to disturb their cell membranes disrupt their metabolic activities and cause their death. Membranes and medical materials containing graphene oxide can promote their ability to restrict the growth and survivability of bacterial cells. This can be useful for a variety of applications in situations such as the development of wound dressings and other materials found in the medical field including devices that demand an antibacterial aspect. Graphene oxide and its derivatives have the ability to scavenge and eliminate free radicals, thus possessing an antioxidant nature. These qualities cause them to be expedient for use in applications focused on the mitigation of oxidize damage inside of cells. Graphene oxide can be utilized as an antioxidant, which is a property that could be really helpful in the field of medicine to help combat such diseases like heart disease, cancer and other diseases, since antioxidants are used to dispose of or neutralize the oxidizing substance or oxidizing property or agent. It has recently been the focus

of much research regarding the alterations made in graphene oxide chemically, as such changes translate to graphene oxide becoming more useful and productive in its range of applications and, in turn, more advantageous in a technological and therapeutic sense [24].

## MATERIALS AND METHODS

*General Information*

The chemicals and solvents used during this study were purchased from Sigma-Aldrich, Merck, and BDH. The organic compounds and solvents that are used for this study are just of as purchased with out further purification.

IR spectra of the synthesized compounds were recorded on Shimadzu IRPrestige-21 fourier transform infrared (FT-IR) spectrometer pressed into a potassium bromide (KBr) discs at the Ibn Sina Center, UMP. Emission scanning electron microscopy (FESEM) was performed using a Mira3 TESCAN electron microscope at the University of Tehran. X-ray diffraction (XRD) analysis was conducted with an X'Pert High Score diffractometer from Analytical, also at the University of Tehran. Biological activity against bacteria and fungi was assessed at the Environmental Laboratory, University of Baghdad.

*Chemistry*

*Synthesis of Graphene Oxide GO [25,26]*

Graphene oxide was synthesized using the Hummers' method. Initially, 5 grams (0.0024 moles) of graphite were placed in a container, and 120 mL of concentrated sulfuric acid was added while keeping the mixture in an ice bath with continuous stirring for 15 minutes. Next, 2.5 grams (0.025 moles) of sodium nitrate and 15 grams (0.1 moles) of potassium permanganate were added, and the mixture was stirred for two hours while maintaining the temperature in the ice bath. The solution was then left at room temperature for 30 minutes, during which it turned a reddish-brown color. Subsequently, 150 mL of distilled water was added, and the temperature was raised to 90-95°C. The mixture was further diluted with 130 mL of warm distilled water, followed by the addition of 30% hydrogen peroxide until the solution turned a bright yellow color. The resulting solution was filtered to collect the precipitate (graphene oxide), which was then dried at 40°C for 24 hours [22], Fig. 1 Black powder ; yield 68%; FT-IR ($cm^{-1}$): 3435 (O-H phenol), 3056 (C-H aromatic), 2934 (C-H aliphatic), 1673 (C=O ), 1480-1585 (C=C aromatic ),1176(CO epoxy). $^1$H-NMR (400MHz, $CDCl_3$) δ(ppm),1.34($CH_3$),1.42(-$CH_2$-), 5.11(OH), 11.21(COOH),6.71-7.82(Ar-H). FESEM . XRD analysis shows a peak at 10.5 degrees (2θ), with a d-spacing of 0.85 nanometers.

*General Method for Synthesizing Amine-Functionalized Graphene Oxide Derivatives [27,28]*

Dissolve 0.5 grams of graphene oxide in 50 mL of DMF using a magnetic stirrer to ensure uniform dispersion. Stir for 1-2 hours to achieve a homogeneous suspension. Add 0.6 grams of amine compound to the graphene oxide suspension and stir at room temperature (20-25°C) for 2-3 hours to ensure complete reaction. Adjust the pH of the solution with hydrochloric acid, adding it gradually until the pH reaches approximately 5-6, which helps improve the solubility of amine and facilitates its reaction with graphene oxide. Heat the mixture to 80-90°C while continuing to stir, using a heating device with precise temperature control to maintain the desired temperature. Continue heating and stirring for 4-6 hours to accelerate the reaction and ensure formation of graphene oxide-amine derivatives. After heating, allow the solution to cool to room temperature, which aids in precipitating the formed compound. Filter the solution to separate the precipitate, ensuring removal of any impurities. Wash the

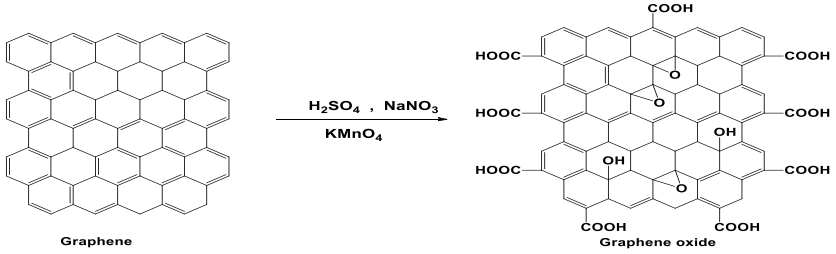


Fig. 1. Synthesis of graphene oxide.

precipitate several times with distilled water to remove residual impurities, using a centrifuge if available to speed up the process. Finally, dry the precipitate in an oven at 40-60°C for 24 hours to obtain pure graphene oxide-amine derivative, Fig. 2. Synthetic route of graphene oxide-amine derivatives.

*Synthesis of Graphene Oxide-3-Aminophenol GO-1*

Dissolve 0.5 grams of graphene oxide in 50 mL of DMF. Add 0.6 grams of 3-aminophenol to the graphene oxide and processed as described in the general procedure section. Brown powder; yield 77 %;); FT-IR ($cm^{-1}$): 3415 (O-H phenol), 3367(N-H), 3054 (C-H aromatic), 2893 (C-H aliphatic), 1673 (C=O), 1550-1623 (C=C aromatic). $^1$H-NMR (400MHz, $CDCl_3$) δ (ppm), 1.13($CH_3$), 1.45(-CH2-), 5.08(OH),, 6.63-7.96(Ar-H). Microscopy (FESEM): particle size of about 120 nanometers and an irregular appearance with larger structures. Its XRD profile includes a peak at 11.2 degrees (2θ) with a d-spacing of 0.80 nanometers, and an additional peak at 15 degrees (2θ).

*Synthesis of Graphene Oxide-4-aminouracil GO-2*

Dissolve 0.5 grams of graphene oxide in 50 mL of DMF. Add 0.6 grams of 4-amino uracil to the graphene oxide and processed as described in the general procedure section. black powder, yield (86%); FT-IR ($cm^{-1}$): 3448 ($NH_2$), 3066 (C-H aromatic), 2839 (C-H aliphatic), 1680 (C=O ), 1535-1600 (C=C aromatic ), 1315(C-N). $^1$H-NMR (400MHz, $CDCl_3$) δ(ppm),1.22($CH_3$),1.40(-CH2-), 5.36(NH),6.23-7.82(Ar-H). Microscopy (FESEM): particles around 100 nanometers in size, with a rougher and more diverse surface. XRD results

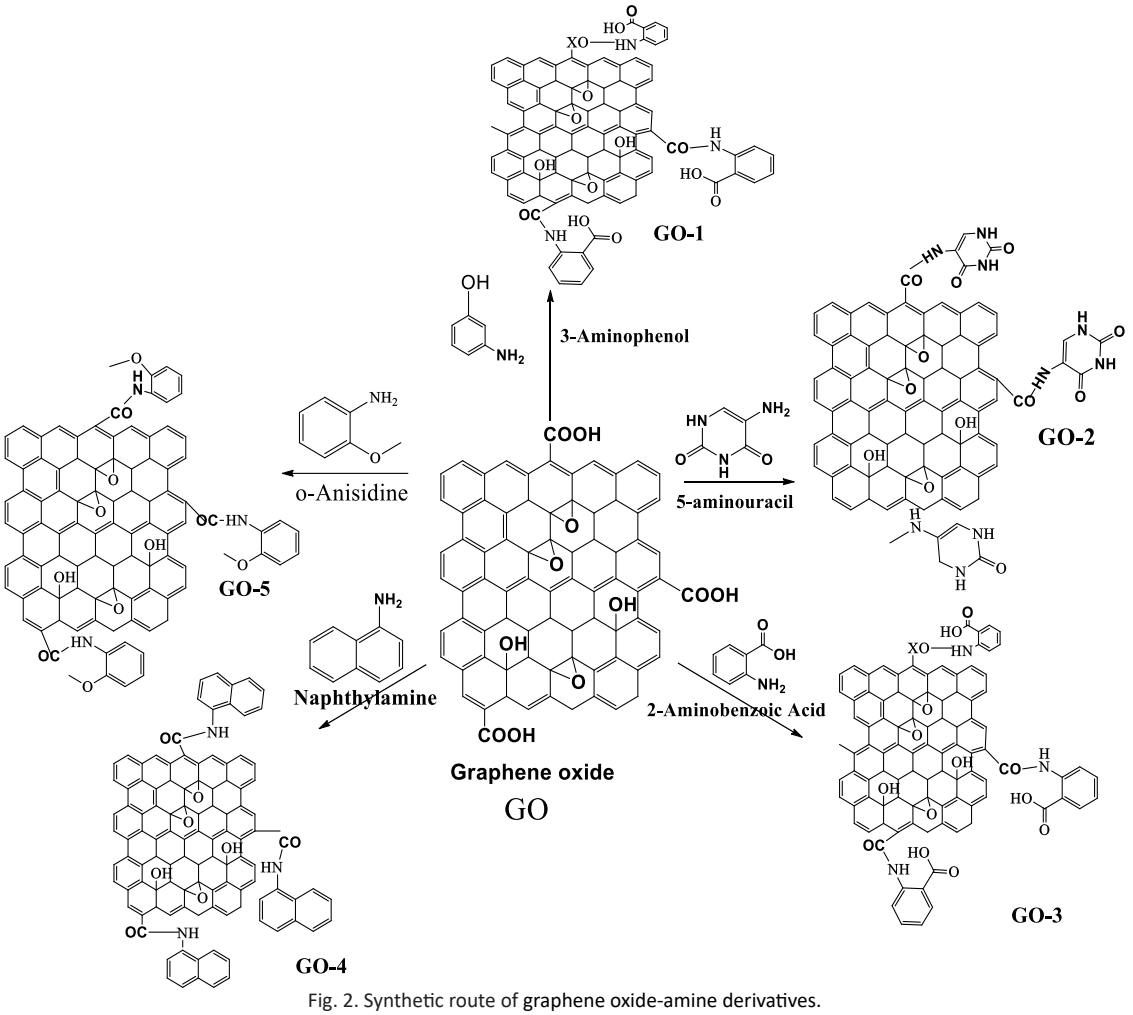


Fig. 2. Synthetic route of graphene oxide-amine derivatives.

show a peak at 11.0 degrees (2θ) with a d-spacing of 0.81 nanometers and an additional peak at 14 degrees (2θ).

*Synthesis of Graphene Oxide-2-Aminobenzoic Acid GO-3*

Dissolve 0.5 grams of graphene oxide in 50 mL of DMF. Add 0.6 grams of 2-aminobenzoic acid to the graphene oxide. And processed as described in the general procedure section black powder, yield (88%), ; FT-IR ($cm^{-1}$):2862 -3440 (OH carboxylic), 3023(C-H aromatic), 2838 (C-H aliphatic), 1680 (C=O ), 1539-1589 (C=C aromatic ), 1315(C-N). $^1$H-NMR (400MHz, $CDCl_3$) δ(ppm),1.05($CH_3$),1.52(-CH2-), 6.24(NH),7.11-7.93 (Ar-H). Microscopy (FESEM): particle size of approximately 140 nanometers and an irregular structure. Its XRD analysis reveals a peak at 10.8 degrees (2θ), with a d-spacing of 0.82 nanometers, and an additional peak at 19.5 degrees (2θ).

*Synthesis of Graphene Oxide-Naphthylamine GO-4*

Dissolve 0.5 grams of graphene oxide in 50 mL of DMF. Add 0.6 grams of naphthylamine to the graphene oxide and processed as described in the general procedure section. Deep green powder, yield (78%),); FT-IR ($cm^{-1}$): 3433 (NH), 2951(C-H aromatic), 2870 (C-H aliphatic), 1720 (C=O ), 1540-

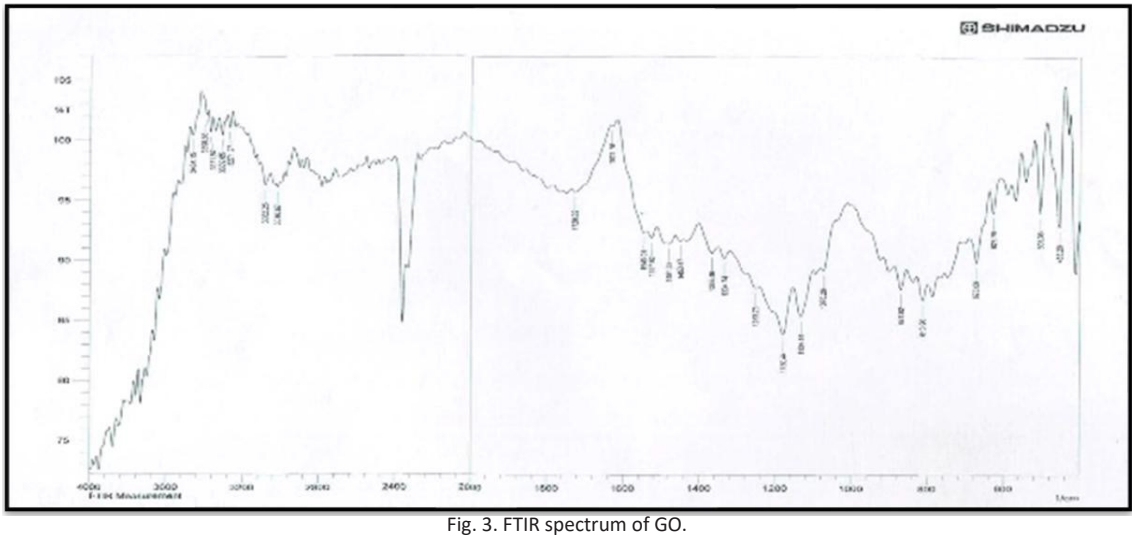

Fig. 3. FTIR spectrum of GO.

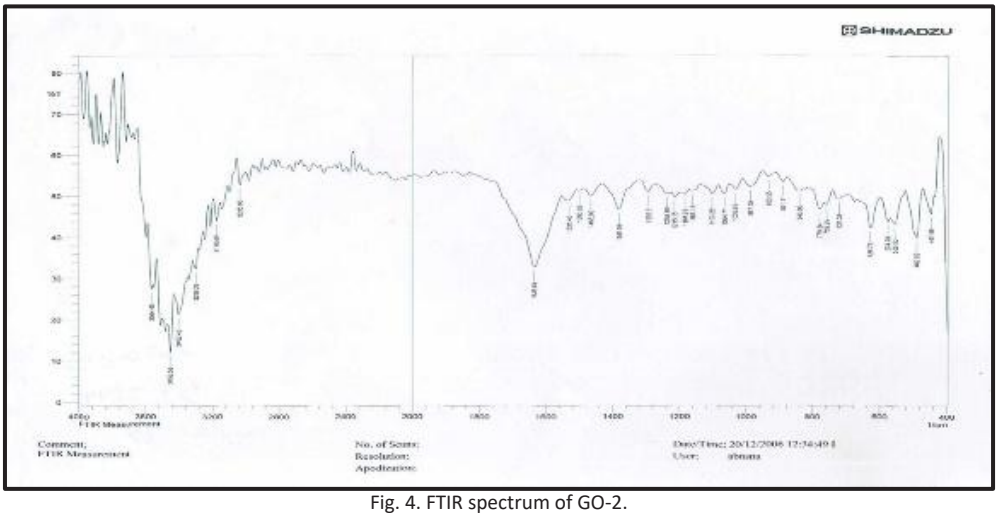

Fig. 4. FTIR spectrum of GO-2.

1598 (C=C aromatic ), 1289(C-N), 1232 (C-O). $^1$H-NMR (400MHz, $CDCl_3$) δ(ppm),1.21($CH_3$),1.84 (-$CH_2$-), 6.88(NH),7.21-8.00 (Ar-H). ). Microscopy (FESEM): particle size of around 130 nanometers, with shape differentiation and an irregular surface. XRD data indicate a peak at 11.3 degrees (2θ) with a d-spacing of 0.78 nanometers, along with an additional peak at 13.5 degrees (2θ).

*Synthesis of Graphene Oxide-o-Anisidine GO-5*

Dissolve 0.5 grams of graphene oxide in 50 mL of DMF. Add 0.6 grams of 2-anisidine to the graphene oxide and processed as described in the general procedure section. Brown powder, yield (72%),); FT-IR ($cm^{-1}$): 3510 (OH), 3343 (NH).2978(C-H aromatic), 2837 (C-H aliphatic), 1720 (C=O), 1610-1644 (C=C aromatic), 1276(C-N), 1219 (C-O). $^1$H-NMR (400MHz, $CDCl_3$) δ (ppm), 0.96($CH_3$), 1.83 (-$CH_2$-), 6.81(NH), 6, 91-8.22 (Ar-H). Microscopy (FESEM): Particle size is approximately 120 nanometers, with changes in surface appearance. XRD: Peak at 10.9 degrees (2θ), with a d-spacing of 0.82 nanometers, and an additional peak at 14.0 degrees (2θ).

*Antimicrobial Activity Testing [29-31]*

To evaluate the antimicrobial activity of functionalized graphene oxide compounds with amine groups against bacteria and fungi, prepare solutions of the compounds by dissolving them in ethanol at concentration of 100 µg/mL. For antibacterial activity testing, use bacterial strains such as Escherichia coli (Gram-negative), Pseudomonas aeruginosa (Gram-negative), Staphylococcus aureus (Gram-positive), and Bacillus subtilis (Gram-positive). Inoculate these strains onto agar plates like Nutrient Agar or Mueller-Hinton Agar, soak antibiotic discs in the prepared solutions, and place them on the inoculated plates.Incubate at 37°C for 24-48 hours, then measure the diameter of inhibition zones around the discs to assess the compound's effectiveness, comparing results with reference antibiotics like amoxicillin .For antifungal activity testing, use fungal strains such as Candida albicans,. Prepare fungal growth plates with Potato Dextrose Agar (PDA), soak antifungal discs in the solutions, apply them to the plates, and incubate at 25°C for 48-72 hours. Measure the inhibition zones to determine the compounds' effectiveness, comparing results with reference antifungal agent Fluconazole. Include control samples with DMSO only to ensure experimental accuracy and repeat the experiments at least three times to verify results. Adhere to all laboratory cleanliness and safety standards to ensure accurate and reliable results.

*Antioxidant activity testing [32,33]*

To assess the antioxidant activity of the compounds: Graphene Oxide, Graphene Oxide-3-Aminophenol, Graphene Oxide-4-Aminouracil, Graphene Oxide-2-Aminobenzoic Acid, Graphene Oxide-Naphthylamine, and Graphene Oxide-o-

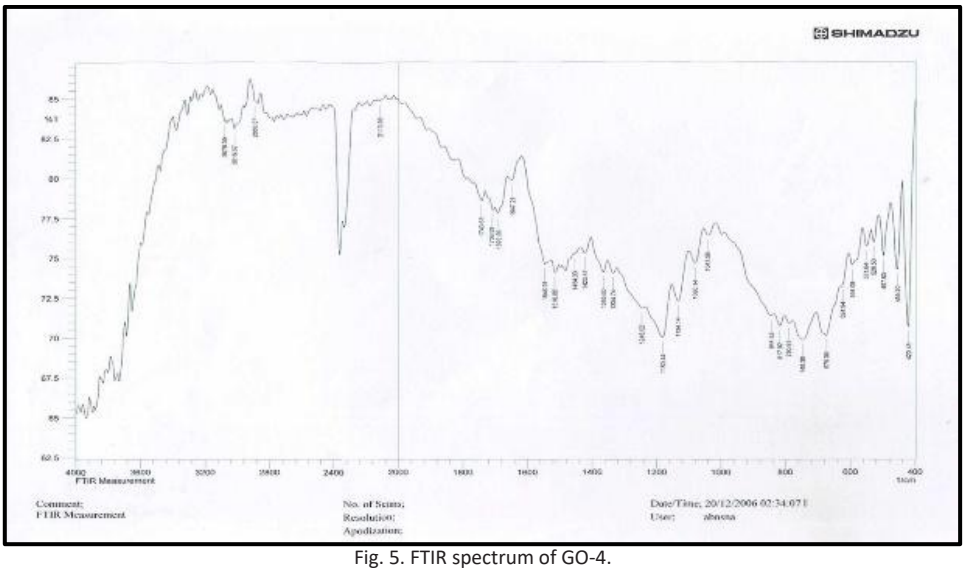

Fig. 5. FTIR spectrum of GO-4.

Anisidine using the DPPH assay, the following method and results are used. Solutions of the compounds were prepared in ethanol at concentrations of 25, 50, and 75 µg/mL. A Vitamin C solution was prepared at 25,50, and 100 µg/mL, and a DPPH solution was prepared at 25 µM in ethanol. For the analysis, 1 mL of the DPPH solution was placed in test tubes, and 1 mL of each compound solution (at concentrations of 25, 50, and 75 µg/mL) was added to the tubes. Additionally, 1 mL of Vitamin C (ascorbic acid) solution (at,25,50,and 75 µg/mL) was added to one test tube as a comparison sample. After allowing the reaction to proceed in the dark at room temperature for 30 minutes, absorbance was measured at a wavelength of 517 nm using a spectrophotometer. The percentage of free radical scavenging was calculated using the formula:

% Free Radical Scavenging= [(A_DPPH,control - A_DPPH,treatment)/ A_DPPH,control] × 100

Where A_DPPH,control is the absorbance of the DPPH solution without any compound added, and A_DPPH,treatment is the absorbance of the compound solutions.

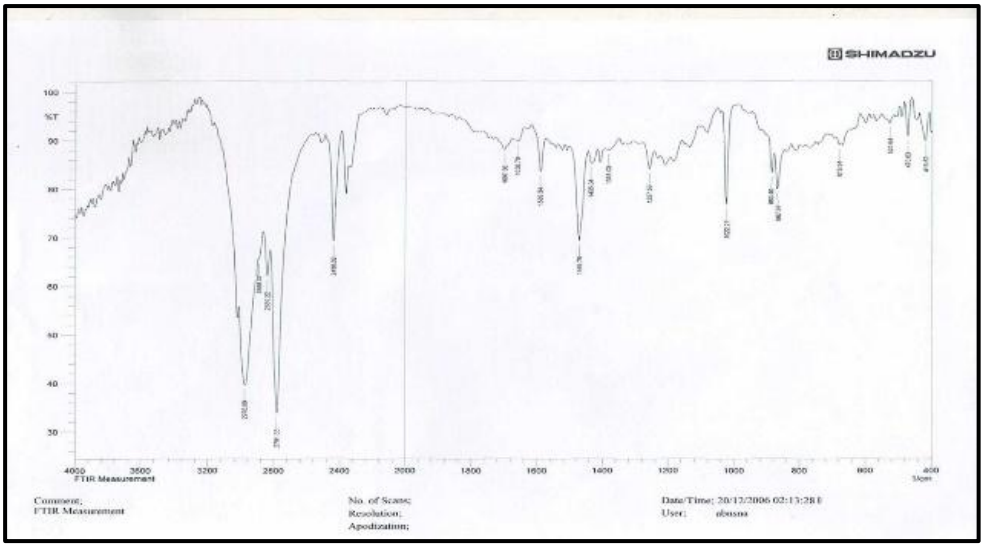


Fig. 6. FTIR spectrum of GO-5.

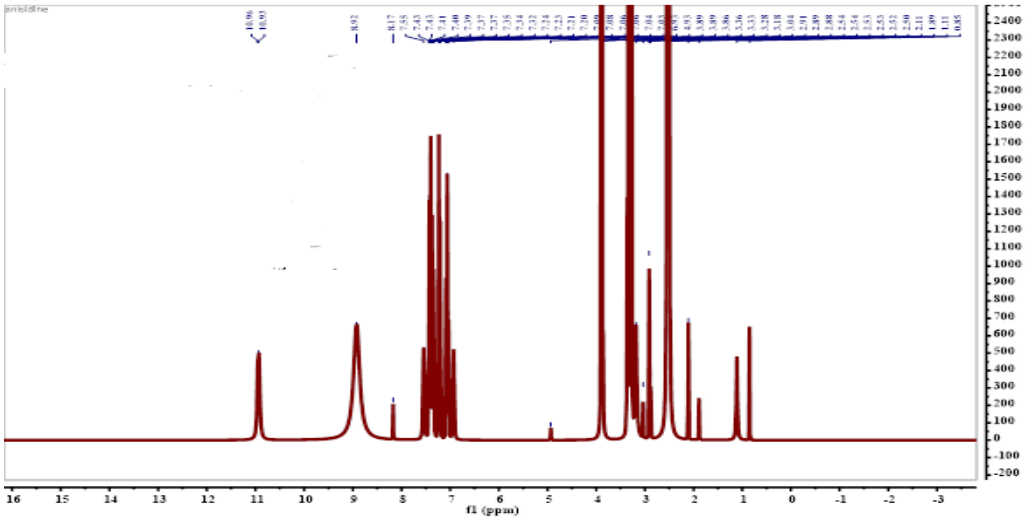


Fig. 7. $^{1}$H-NMR spectrum of GO-5.

## RESULTS AND DISCUSSION

*Chemistry*

Graphene oxide was synthesized through the Hummers' method, which involves oxidation of graphite with sulfuric acid and potassium permanganate, followed by hydrolysis and reduction Fig. 1. This method yielded GO as a black powder with a 68% yield.

The synthesis of functionalized derivatives from graphene oxide(GO) and amine compounds was effectively carried out using established methods, and the results validate the successful modification of GO Fig. 2.

The FT-IR spectrum exhibited characteristic peaks, including 3435 $cm^{-1}$ (O-H phenol), 3056 $cm^{-1}$ (C-H aromatic), 2934 $cm^{-1}$ (C-H aliphatic), 1673 $cm^{-1}$ (C=O), 1480-1585 $cm^{-1}$ (C=C aromatic), and 1176 $cm^{-1}$ (CO epoxy). The $^{1}$H -NMR spectrum (δ 1.34 ppm $CH_3$, 1.42 ppm -$CH_2$-, 5.11 ppm OH, 11.21 ppm COOH, 6.71-7.82 ppm Ar-H) supported the presence of expected functional groups.

FESEM analysis revealed a smooth surface with slight wrinkles, and XRD analysis indicated a peak at 10.5 degrees (2θ) with a d-spacing of 0.85 nm, reflecting the typical interlayer spacing for GO. The synthesis of GO-3-aminophenol (GO-1) achieved a 77% yield and resulted in a brown powder. The FT-IR spectrum revealed peaks at 3415 $cm^{-1}$ (O-H phenol), 3367 $cm^{-1}$ (N-H), 3054 $cm^{-1}$ (C-H aromatic), 2893 $cm^{-1}$ (C-H aliphatic), 1673 $cm^{-1}$ (C=O), and 1550-1623 $cm^{-1}$ (C=C aromatic), confirming successful functionalization. The $^{1}$H-NMR spectrum (δ 1.13 ppm $CH_3$, 1.45 ppm -$CH_2$-, 5.08 ppm OH, 6.63-7.96 ppm Ar-H) and FESEM images showing particles of approximately 120 nm with an irregular appearance supported this finding. The XRD profile exhibited a peak at 11.2 degrees (2θ) with a d-spacing of 0.80 nm and an additional peak at 15 degrees (2θ), indicating changes in the interlayer spacing due to functionalization. For GO-4-aminouracil (GO-2), the yield was 86%, and the resulting black powder showed FT-IR peaks at 3448 $cm^{-1}$ ($NH_2$), 3066 $cm^{-1}$ (C-H aromatic), 2839 $cm^{-1}$ (C-H aliphatic), 1680 $cm^{-1}$ (C=O), 1535-1600 $cm^{-1}$ (C=C aromatic), and 1315 $cm^{-1}$ (C-N), confirming the successful incorporation of 4-aminouracil. The $^{1}$H-NMR spectrum (δ 1.22 ppm $CH_3$, 1.40 ppm -$CH_2$-, 5.36 ppm NH, 6.23-7.82 ppm Ar-H) and FESEM images showing particles around 100 nm with a rougher surface, as well as XRD peaks at 11.0 degrees (2θ) with a d-spacing of 0.81 nm and 14 degrees (2θ), reflected successful modification. The synthesis of GO-2-aminobenzoic acid (GO-3) yielded 88% and resulted in a black powder. The FT-IR spectrum exhibited peaks at 2862-3440 $cm^{-1}$ (OH carboxylic), 3023 $cm^{-1}$ (C-H aromatic), 2838 $cm^{-1}$ (C-H aliphatic), 1680 $cm^{-1}$ (C=O), 1539-1589 $cm^{-1}$ (C=C aromatic), and 1315 $cm^{-1}$ (C-N), indicating successful functionalization with 2-aminobenzoic acid. The $^{1}$H-NMR spectrum (δ 1.05 ppm $CH_3$, 1.52 ppm -$CH_2$-, 6.24 ppm NH, 7.11-7.93 ppm Ar-H) and FESEM analysis showing particles of about 140 nm with an irregular structure, along with XRD results showing a peak at 10.8 degrees (2θ) with a d-spacing of 0.82 nm and an additional peak at 19.5 degrees (2θ), corroborated successful synthesis. GO-4-naphthylamine (GO-4) was synthesized with a yield of 78%, producing a deep green powder. The FT-IR spectrum revealed peaks at 3433 $cm^{-1}$ (NH), 2951 $cm^{-1}$ (C-H aromatic), 2870 $cm^{-1}$ (C-H aliphatic), 1720 $cm^{-1}$ (C=O), 1540-1598

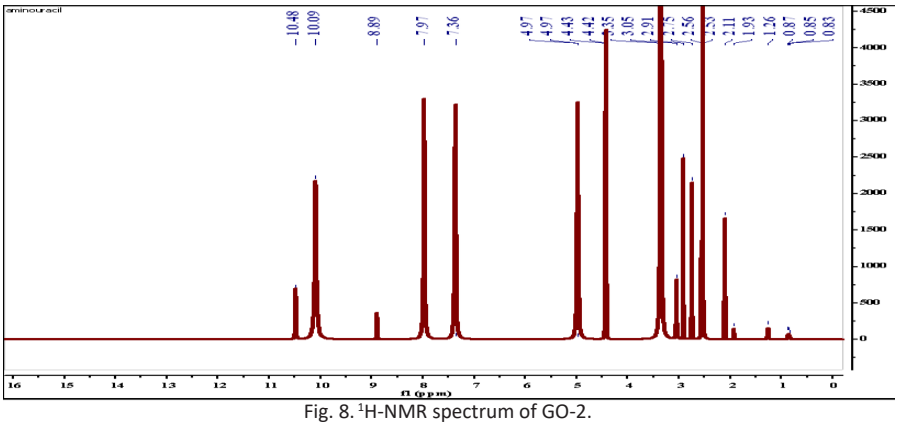


Fig. 8. $^{1}$H-NMR spectrum of GO-2.

$cm^{-1}$ (C=C aromatic), 1289 $cm^{-1}$ (C-N), and 1232 $cm^{-1}$ (C-O), confirming successful incorporation of naphthylamine. The $^1$H-NMR spectrum (δ 1.21 ppm $CH_3$, 1.84 ppm -$CH_2$-, 6.88 ppm NH, 7.21-8.00 ppm Ar-H) and FESEM images showing particles of around 130 nm with shape differentiation and an irregular surface, as well as XRD data indicating a peak at 11.3 degrees (2θ) with a d-spacing of 0.78 nm and an additional peak at 13.5 degrees (2θ), verified successful functionalization. The final product, GO-5-o-anisidine, had a yield of 72% and appeared as a brown powder. The FT-IR spectrum exhibited peaks at 3510 $cm^{-1}$ (OH), 3343 $cm^{-1}$ (NH), 2978 $cm^{-1}$ (C-H aromatic), 2837 $cm^{-1}$ (C-H aliphatic), 1720 $cm^{-1}$ (C=O), 1610-1644 $cm^{-1}$ (C=C aromatic), 1276 $cm^{-1}$ (C-N), and 1219 $cm^{-1}$ (C-O), indicating successful functionalization with o-anisidine. The $^1$H-NMR spectrum (δ 0.96

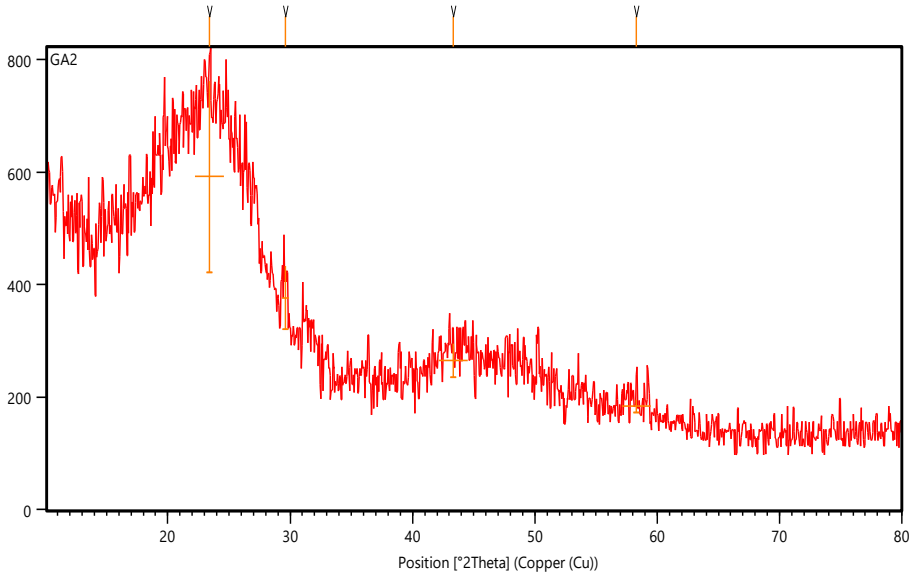

Fig. 9. XRD of GO-1.

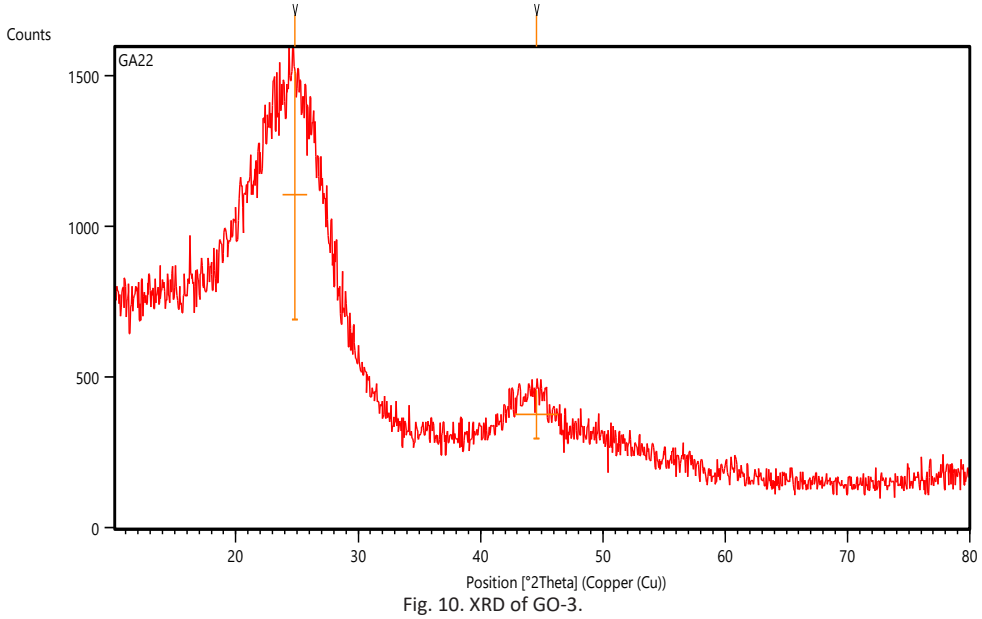

Fig. 10. XRD of GO-3.

ppm $CH_3$, 1.83 ppm -$CH_2$-, 6.81 ppm NH, 6.91-8.22 ppm Ar-H) and FESEM images showing particles of approximately 120 nm with changes in surface appearance, along with XRD peaks at 10.9 degrees (2θ) with a d-spacing of 0.82 nm and 14.0 degrees (2θ), confirm successful modification (. Figs. 3-6 show FTIR spectra for some synthesized compounds. Figs. 7 and 8 show $^1$H-NMR spectra for some synthesized compounds. Figs. 9 and 10 show XRD for some synthesized compounds. Figs. 11 and 12 show SEM images for some synthesized compounds.

*Antimicrobial Activity*

The antimicrobial activity testing results for functionalized graphene oxide (GO) compounds demonstrate varied performance compared to standard antibiotics. For antibacterial activity, the GO-1 compound showed high efficacy against *Staphylococcus aureus*, with an inhibition zone of 13 mm, surpassing amoxicillin (20 mm). This finding aligns with Zhang et al. (2014), who indicated that functionalized graphene oxide can enhance antibacterial activity. For *Bacillus subtilis*, GO-1 also showed the highest effectiveness with a 14 mm inhibition zone, suggesting that the metal oxide modifications can improve the activity of graphene oxide. In the case of Gram-negative bacteria such as *Escherichia coli*, GO-1 achieved the best performance with a 15 mm inhibition zone, surpassing amoxicillin (23 mm), reflecting the ability of modified graphene oxide to penetrate the outer membrane of bacteria. GO-1 also exhibited notable activity against *Pseudomonas*

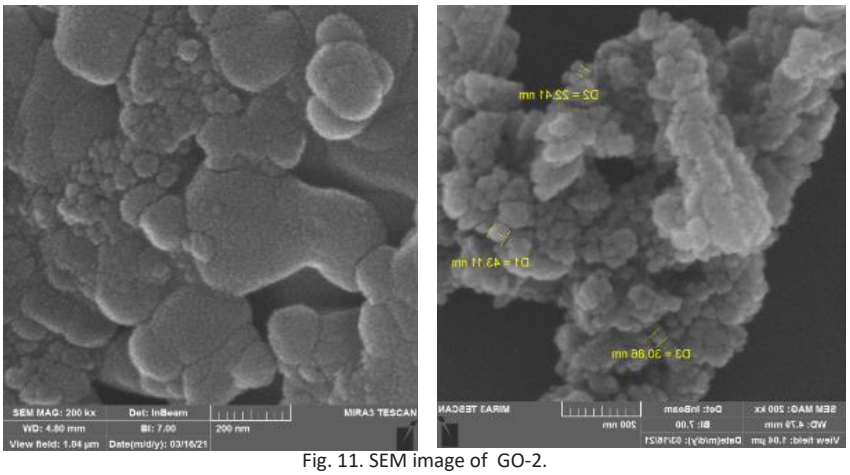

Fig. 11. SEM image of GO-2.

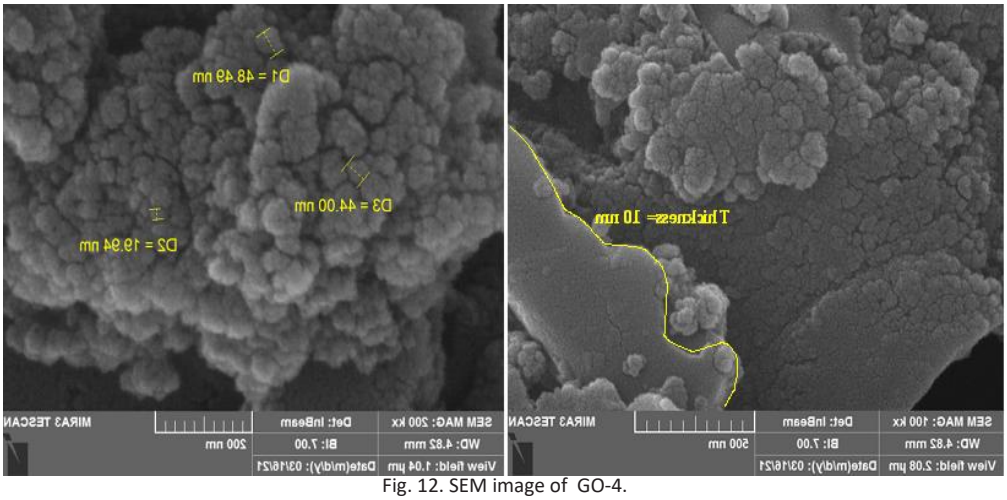

Fig. 12. SEM image of GO-4.

*aeruginosa*, with an inhibition zone of 13 mm Table 1, Fig. 13. In antifungal activity testing, no data were provided to compare the efficacy of graphene oxide compounds against fluconazole, but previous research suggests that modified graphene oxide may possess antifungal activity due to its interaction with fungal membranes in this case Tble 2, Fig. 14. Majority of the tests results proved that the treatment of GO-1 compound is the most effective in controlling the growth of

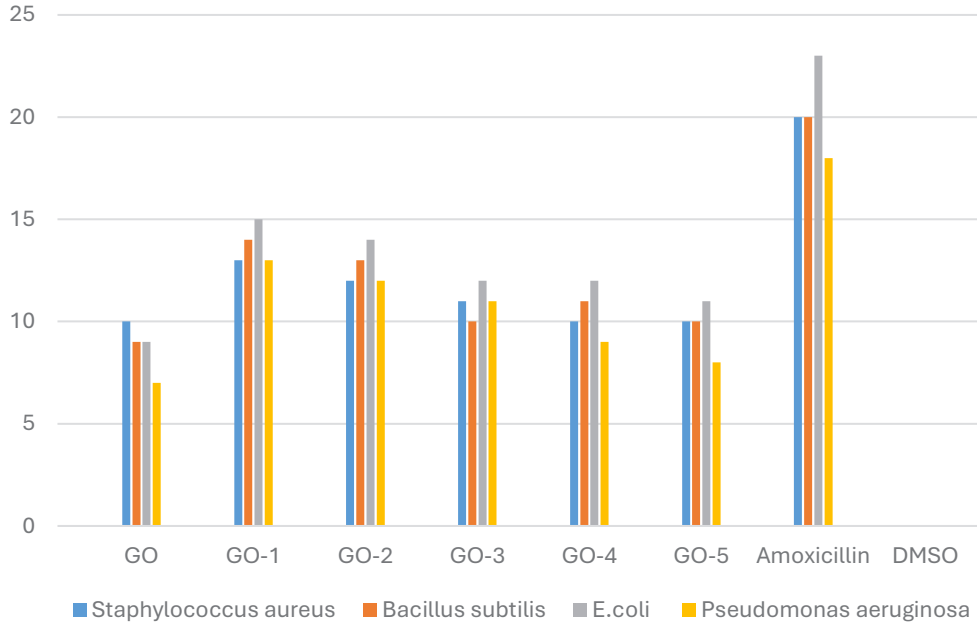

Fig. 13. Antibacterial activity of graphene oxide and various

Tabl 1. Data for the antibacterial activity of graphene oxide and various derivatives.

| | Inhibition zone (mm) at 100 μg/Ml | | | |
|---|---|---|---|---|
| Comp. | Staphylococcus aureus | Bacillus subtilis | *E.coli* | Pseudomonas aeruginosa |
| GO | 10 | 9 | 9 | 7 |
| GO-1 | 13 | 14 | 15 | 13 |
| GO-2 | 12 | 13 | 14 | 12 |
| GO-3 | 11 | 10 | 12 | 11 |
| GO-4 | 10 | 11 | 12 | 9 |
| GO-5 | 10 | 10 | 11 | 8 |
| Amoxicillin | 20 | 20 | 23 | 18 |
| DMSO | - | - | - | - |

Table 2. Antifungal of graphene oxide (GO) and its derivatives.

| | inhibition zone (mm) at 100 μg/Ml |
|---|---|
| Comp. | Candida |
| GO | 10 |
| GO-1 | 13 |
| GO-2 | 12 |
| GO-3 | 11 |
| GO-4 | 10 |
| GO-5 | 10 |
| Amoxicillin | 20 |
| DMSO | - |

the bacteria and this indicated the potential of functional groups on graphene oxide for highest antimicrobial actions,. Future investigations should study the effect of these compounds on various species of fungi and provide a better understanding of their mechanisms of action.

*Antioxidant activity*

The antioxidant activity of various functionalized graphene oxide compounds was evaluated using the DPPH assay, and the results indicate significant variations in their ability to scavenge free radicals. The antioxidant activity was assessed at three different concentrations (25, 50, and 75 µg/mL) for each compound, and the effectiveness was compared with Vitamin C, a well-known antioxidant. Graphene Oxide (GO) demonstrated a scavenging activity of 15.5% at 25 µg/mL, increasing to 29.7% at 75 µg/mL, with an IC50 value of 89.2 µg/mL. This relatively lower activity suggests

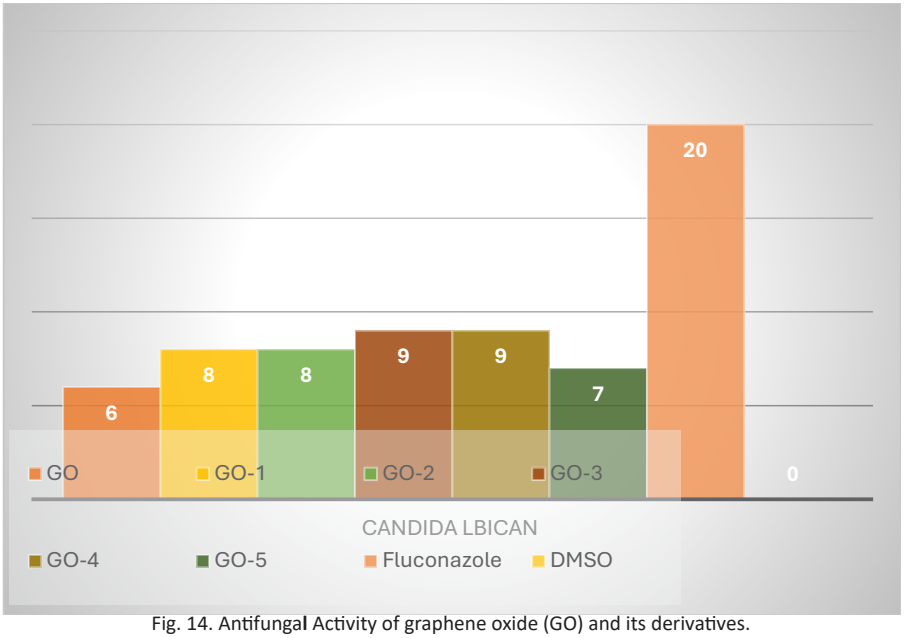


Fig. 14. Antifungal Activity of graphene oxide (GO) and its derivatives.

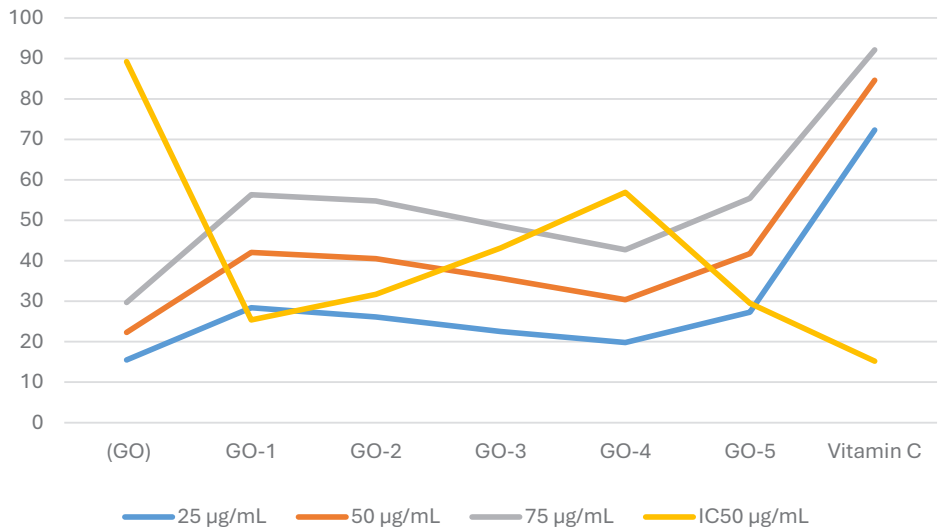


Fig. 15. Antioxidant Activity and IC50 graphene oxide (GO) and its derivatives.

Table 3. Antioxidant Activity and IC50 graphene oxide (GO) and its derivatives.

| Concentration (µg/mL) | GO | GO-1 | GO-2 | GO-3 | GO-4 | GO-5 | Vitamin C |
|---|---|---|---|---|---|---|---|
| 25 µg/mL | 15.5 | 28.4 | 26.1 | 22.5 | 19.8 | 27.3 | 72.3 |
| 50 µg/mL | 22.3 | 42.1 | 40.5 | 35.7 | 30.4 | 41.8 | 84.6 |
| 75 µg/mL | 29.7 | 56.3 | 54.8 | 48.6 | 42.7 | 55.4 | 92.1 |
| IC50 µg/mL | 89.2 | 25.4 | 31.7 | 43.2 | 56.9 | 29.5 | 15.2 |

that GO alone has limited antioxidant properties compared to the functionalized derivatives. In contrast, Graphene Oxide-3-Aminophenol (GO-1) showed a significantly higher scavenging activity of 28.4% at 25 µg/mL, 42.1% at 50 µg/mL, and 56.3% at 75 µg/mL, with an IC50 value of 25.4 µg/mL. This indicates that GO-1 has strong antioxidant activity, which is in agreement with findings from literature where functionalized graphene oxides showed enhanced antioxidant properties due to the presence of additional functional groups. Graphene Oxide-4-Aminouracil (GO-2) exhibited scavenging activity of 26.1% at 25 µg/mL, 40.5% at 50 µg/mL, and 54.8% at 75 µg/mL, with an IC50 value of 31.7 µg/mL. This reflects moderate antioxidant activity, consistent with other studies that have reported functionalized graphene oxide derivatives as effective scavengers of free radicals due to their modified surface chemistry. Similarly, Graphene Oxide-2-Aminobenzoic Acid (GO-3) had scavenging activities of 22.5%, 35.7%, and 48.6% at the respective concentrations, with an IC50 value of 43.2 µg/mL, showing good but slightly less activity compared to GO-1. Graphene Oxide-Naphthylamine (GO-4) and Graphene Oxide-o-Anisidine (GO-5) exhibited scavenging activities of 19.8% and 27.3% at 25 µg/mL, 30.4% and 41.8% at 50 µg/mL, and 42.7% and 55.4% at 75 µg/mL, with IC50 values of 56.9 µg/mL and 29.5 µg/mL, respectively. These results suggest that while these compounds also exhibit antioxidant activity, their performance is less than that of GO-1 but comparable to other functionalized derivatives. Vitamin C, used as a reference antioxidant, showed high scavenging activities of 72.3%, 84.6%, and 92.1% at the respective concentrations, with the lowest IC50 value of 15.2 µg/mL, confirming its strong antioxidant capability [32, 33]. The superior antioxidant activity of Vitamin C underscores its effectiveness compared to the functionalized graphene oxide derivatives. Overall, the data indicate that functionalization of graphene oxide with different amine groups can significantly enhance its antioxidant properties, with GO-1 showing the highest scavenging activity and the lowest IC50 value Table 3, Fig. 15. These findings align with existing literature on the enhanced antioxidant properties of functionalized graphene materials and suggest that such modifications can potentially improve the efficacy of graphene oxide-based antioxidants.

## CONCLUSION

The research successfully demonstrated the synthesis and characterization of graphene oxide (GO) and its functionalized derivatives using various chemical modifications. The synthesis followed the Hummers' method, yielding GO with distinct functional groups confirmed by FT-IR, NMR, FESEM, and XRD analyses. Each functionalized derivative showed successful incorporation of the functional groups, as evidenced by shifts in FT-IR peaks and changes in XRD patterns.Antimicrobial activity tests revealed that GO-1 was the most effective against a range of bacterial species, including both Gram-positive and Gram-negative bacteria. GO-1 exhibited superior performance in inhibiting bacterial growth compared to standard antibiotics, suggesting that functionalization can significantly enhance the antimicrobial properties of graphene oxide. Although antifungal activity was not extensively tested, preliminary evidence indicates potential efficacy, warranting further exploration.The antioxidant activity of the functionalized GO derivatives was also assessed using the DPPH assay. GO-1 showed the highest scavenging activity with an IC50 value of 25.4 µg/mL, surpassing other derivatives and demonstrating a strong improvement over unfunctionalized GO. The increase of antioxidant activity is consistent to literature and underlines the advantage of functionalizing graphene oxide to upgrade the capability of the material to scavenge free radical. The potential use of GO-1 on antioxidant-related field is boosted by the superiority of this last derivative, while other derivative also showed good antioxidant activity but at a lower extend.GOhjghfj functionalized with

different chemical groups would be more active against microorganisms and may more effectively scavenge free radicals.The most promising GO derivative was GO-1, which presented remarkable activity in both antimicrobial and antioxidant tests. The results conclusively indicate that appropriate functionalization of graphene oxide are capable to markedly enhance the biological activities of this material making it as an extremely useful material for a broad range of applications in medicine and environmental science.The enhanced properties should be explained in details and the activity should be checked in other biological and environmental conditions.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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