RESEARCH ARTICLE

Synthesis and Characterization of New Graphene Oxide Nano Derivatives, and study of their Biological Activities

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ABSTRACT

Preparation of graphene oxide and react it with many compounds by forming an amide group because graphene oxide (GO) Its molecular structure is saturated with carboxylic groups, making it an ideal for esterification and amidation.We exploit graphene oxide's capacity to interact with many compounds because of the carbonyl, hydroxyl, and epoxide functional groups in GO to create new products by reacting graphene oxide with those compounds (2-aminopyrmidine, dithiazone, sulfamide, and 1-amino-2-naphthol-4sulfonic acid). Using fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR), field emission scanning electron microscope (FESEM), and X-ray diffraction were used to characterize the derivatives. The X-ray diffraction (XRD) pattern was estimated using two-particle size equations and compared between them. Furthermore, studying the antimicrobial activities of graphene oxide derivatives, inhibition zone illustrates the potency of compound on bacteria and fungi.

Keywords: Amidation, Derivatives, Graphene Oxide, XRD.

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INTRODUCTION

A single layer of graphite is called a Graphene, where sp2 bonded carbon atoms are arranged in a 2D hexagonal frame first reported in 2004.[1] Because of its unique structural, physicochemical, and mechanical properties, graphene has gotten much recognition.[2] On both sides of a single graphite sheet, a high number of oxygen-containing functional groups have been added (namely, graphene). The implantation of functional groups overcomes the van der Waals force between the sheets and increases interlayer separation.[3]

Graphene is a fascinating substance.[4] It has a large real-surface area theoretically(a2630 m^2 g^{-1}), moderate intrinsic mobility (200,000 cm^2 v^{-1} s^{-1}), and high intrinsic mobility (200 000 cm^2 v^{-1} s^{-1}),[5]as well as the optical transmittance (97.7%) For applications such as transparent conductive electrodes, and good electrical conductivity are essential.[6]

Graphene's carbon backbone can be modified or functionalized in an almost infinite number of ways, making it a versatile and robust membrane.[7] While the specific composition of graphene oxide is unknown, it is apparent that carboxylic groups, epoxides, ketone, alcohols,carbonyls, and disrupt the previously contiguous aromatic lattice of graphene.[8]

The unique properties of graphene are the primary explanation for the rapid growth of graphene science. Several experimentally measured properties have also outperformed those of other compounds. Electrical conductivity is improved, mechanical strength is increased, thermal conductivity is increased, and gas impermeability is increased, graphene has much potential as the following great material.

Graphene oxide (GO) and reduced graphene oxide (rGO) are two main types of graphenethathave attracted numerous scientific sectors due to their extraordinary features, such as large surface area, amazing electrical and thermal conductivities, solid mechanical power, and optical clarity.[9-13]

While the exact composition of GO cannot be exactly determined, epoxides, alcohols, ketones, and carboxylic groups appear to destabilize graphene's previously infectious aromatic lattice. The lattice has been fractured when interlayer spacing increases from 0.335 nm for graphite to more than 0.625 nm for GO.[8,14]

The modified Hummers technique was utilized to oxidize the GO, which was subsequently thermally reduced to generate graphene in this work. GO is produced by oxidative treatment of graphite using one of the primary methods proposed by Brodie, Hummers, or Staudenmaeir, which offers numerous benefits over existing reduction processes. To begin, the response should be completed within a few hours. Then, instead of $KMnO_4$, $KClO_3$ was employed to increase reaction protection by avoiding explosive ClO_2 development. $KClO_3$ is a chemical compound with the formula $KClO_3$. Finally, using

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$NaNO_3$ instead of HNO_3 does not result in the formation of fuming acid fog.[15,16]

EXPERIMENTAL PART

Synthesis of Graphene Oxide GO

According to Hummers method, Graphene oxide was prepared.[18,19] In this technique, we used 2 g of graphene and added to cool 50 mL of concentrated H_2SO_4 for 15 minutes stirring in an ice bath. To the above solution, 1-g sodium nitrate and 6 g potassium permanganate in an ice bath were added and stirred for 2 hours. The icy bath was taken away, and the mixture was held in a water bath for 30 minutes at 35°C. The mixture got pasty then after (deep red-browncolor). Above that, the mixture was then given 50 mL of deionized water. The temperature then rose to between 90 and 98 degrees Celsius. 250 mL warm deionized water was added to the above mixture to dilute it. The solution was then treated with 30 mL of 30% H_2O_2 soon, and then it became bright yellow. The graphene oxide powder was dried at 40 degrees Celsius for 24 hours.

Synthesis of GO-2-aminopyrimidine

Weight 0.1 gm graphene oxide and dissolved it in 100 mL of DMF, then add 0.1 g 2-aminopyrmidine to the solution of Dissolved GO. In a tiny amount of water, dissolve sodium nitrate and add it to the beaker. Then beaker was then placed in the ultrasonic power 410 for 30 minutes. Next, thebeaker was then positioned on the hotplate and stirred for 24 hours at 45 degrees Celsius.[19,20]

A 2-mL of HCl was gradually added to the mixture, drop by drop after 24 hours of stirring, until the 2 mL ends the sample was eventually purified with filter paper (black ppt).[22,23]

Synthesis of GO-1-2-naphthol4-sulfonic Acid

Weight 0.1 gm of graphene oxide and 100 mL of dimethylformamide (DMF), then add the sample 1-2-naphthol-4-sulfonic acid. Dissolve sodium nitrate in water (smallest amount possible) and added it to the beaker. Next we put the beaker into the ultrasonic (sonic power410) for 30 minutes then, we put the beaker which is covered with parafilm on the hotplate and stirrer for 24 hours with 45ºC.

After 24 hours of stirring 2 mL of HCl was added gradually to the mixture drop by drop finally we filter the sample with filter paper (dark purple ppt).[24-26]

Synthesis of GO-sulfamide

Weight 0.1 gm of graphene oxide and 100 mL of DMF then add the sample 0.5 mL of sulamide. Dissolve sodium nitrate in water (smallest amount possible) and added it to the beaker black color solution appeared. next we put the beaker into the ultrasonic power 410 for 30 minutes. Then, we put the beaker which covered with parafilm on the hotplate and stirrer for 24 hours with 45ºC.

After 24 hours, 45ºC temperature of stirring 1-2 mL of HCl was added gradually to the mixture drop by drop finally we filter the sample with filter paper (black ppt).[22,23,27,28]

Synthesis GO-dithiazone

Weight 0.1 gm of graphene oxide and 100 mL of DMF then add the sample 0.5 mL of Dithiazone. Dissolve sodium nitrate in water (smallest amount possible) and added it to the beaker dark green solution appeared. Next, we put the beaker into the ultrasonic (sonic power 410) for 30 minutes and turn the color from dark green into dark brown. then, we put the beaker which is covered with parafilm on the hotplate and stirrer for 24 hours with 45ºC.

After 24 hours and 45ºC temperature of stirring 1 to 2 mL of HCl was added gradually to the mixture drop by drop finally, we filter the sample with filter paper (dark brown ppt).[29,30]

RESULTS AND DISCUSSION

FTIR Spectroscopy

H^1NMR Spectroscopy

H^1NMR for GO

The characteristic peaks at (8.08) ppm in the H1-NMR spectra of graphene oxide are attributed to COOH groups' proton (GO).

Table 1: The FTIR ν (cm^{-1}) spectral data for graphene oxide and its derivatives

Comp. No.	*ν (O-H)*	*ν(N-H)*	*ν(C-H) Ar.*	*ν(C-H) Aliph.*	*ν(C=N)*	*ν (C-N)*	*ν(C=C) Ar.*	*ν (C-O) epoxy*	*Others*
1 (24)(25)(26).	3383.44	3367.71	3251.98	3209.55	–	–	1622.8	1029.8	(C=O) 1724.36 (C-O) 1361.74
2 (27).	3433.29	3390,86	3367.71	3309.85	1550.77	1087.85	1500.62	1045.42	(C=O) 1627.92 ν(SO2) sym. 1184.29 ν(SO2) asym. 1253.73
3 (27)(28).	3464.15	3390.86	3059	2943	1438.90	1134.14	1545.05	1083.99	(C=O) 1631.76
4 (30).	3549.02	3414	3093	2920.23	1616.35	1130.29	1543.05	1384.89	ν(SO2) sym. (1180.44) (C=O) 1708
5 (27)(29).	3549.02	3468.01	3298.28	3240.41	1465.90	1087.85	1539.20	1022.27	(C=S) 1184.29. (C=O) 1639.49

* Compound 1 = Graphene oxide, Compound 2= GO-1-amino-2-naphthol-4sulfonic acid, Compound 3= GO-2-aminopyrimidine, Compound 4= GO-sulfamide, Compound 5= GO-dithiazone

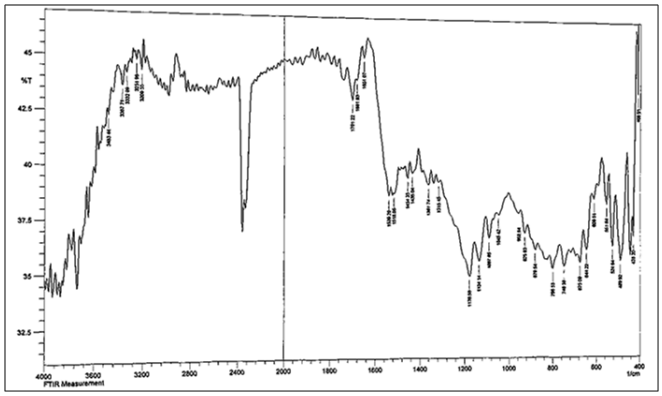


Figure 1: FTIR of graphene oxide

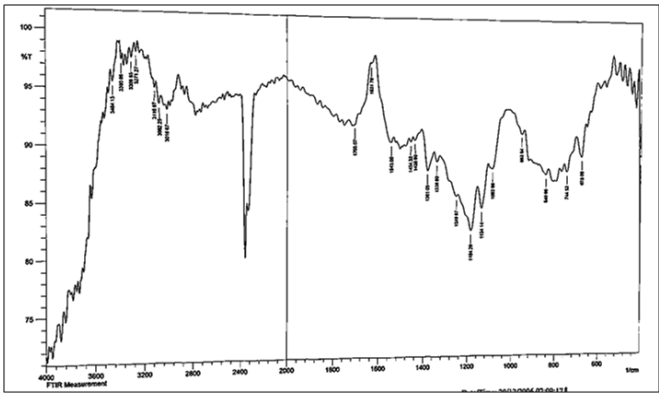


Figure 2: FTIR of GO-2-aminopyrimidine

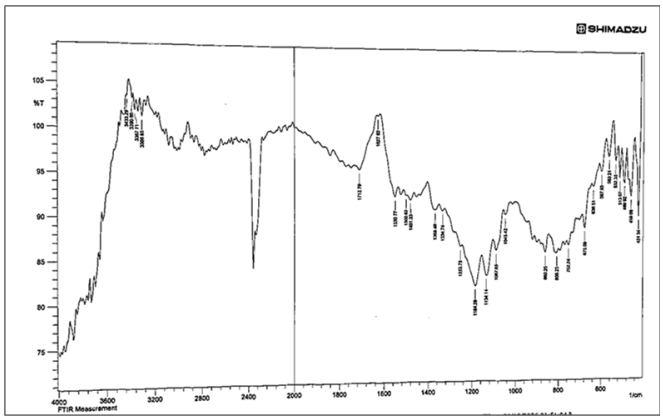


Figure 3: FTIR of GO-1-amino-2-naphthol-4-sulfonic acid.

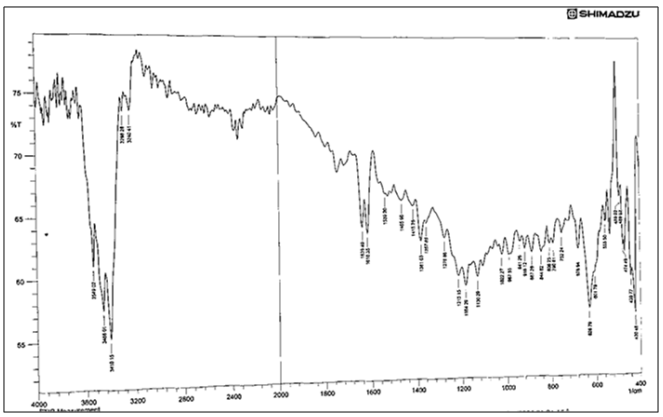


Figure 4: FTIR of GO-dithiazone

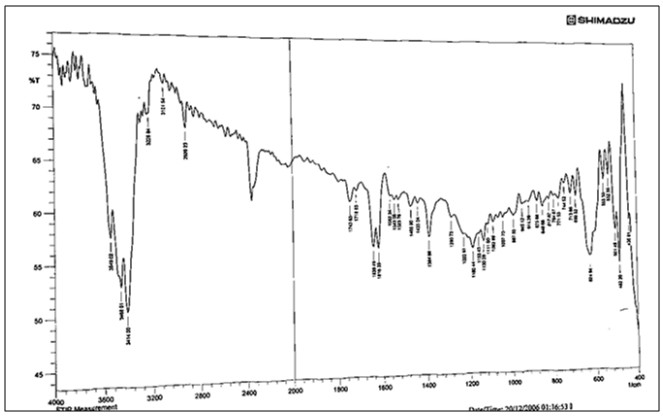


Figure 5: FTIR of GO-sulfamide

The protons of OH phenolic groups are allocated a singlet at (7.50) ppm, whereas phenyl groups are allocated a singlet at (7) ppm.Because hydrogen bonding between the layers of GO graphene oxide Deshields the electron density, all of them are weak and broad, resulting in a decrease in the chemical shift of the protons. The singlet is present in the solvent at a frequency of (2.5) ppm.The CH groups, which also produce a chemical shift, are singlets assigned to the (3.75) ppm peak.[21,24,31-35]Table 2 shows the data of HNMR for graphene oxide and its derivatives.

(XDR) X-Ray Diffraction of GO

XRDOF GO

The X-Ray Diffraction (XRD) of grapheme oxide reveals a wide interlayer spacing of 8.07 Aº at position (2 = 10.98˚) with the peak at 27º disappearing due to complete oxidation after chemical oxidation and exfoliation.[18]

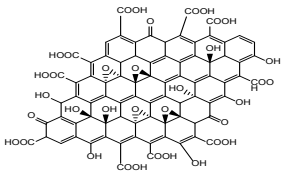
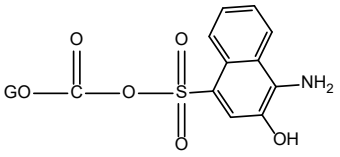
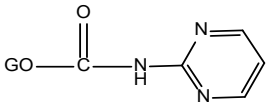
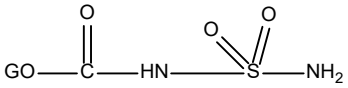
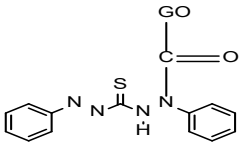
XRD of GO-1-amino-2-naphthol-4sulfonic acid, GO-sulfamide, and GO-dithiazone

X-ray diffraction measurements of the nano compounds (GO-1-amino-2-naphthol-4sulfonic acid), (GO-sulfamide), and (GO-dithiazone) showed completely similar patterns with very little difference in the 2 theta and FWHM values. As follows: the first compound (GO-1-amino-2-naphthol-4sulfonic acid) (Figure 6) showed the following peaks:

22.7351, 24.8674, and 42.8817° while the compound (GO-sulfamide) (Figure 7) showed the following peaks: 22.8241, 24.9153, 31.7969, 43.2487, and 45.6493°. Finally, the compound (GO-dithiazone) (Figure 8) showed the following peaks: 23.0434, 25.4229, 31.7989, 43.3356, and 45.6308°.[36]

The disappearance of the characteristic peak of graphene oxide, which appears at approximately 10°, as well as the appearance of the broad peak in the range 22.7–23.04°, is evidence that these amines performed a reduction of the

Table 2: Data of HNMR spectra of graphene oxide and graphene oxide derivatives.

Comp. No.	*Comp. structure*	*^{1}H-NMR parameters (δppm)*
1		(8.08) ppm =COOH, (7.50) ppm= OH phenolic groups, phenyl groups singlet at= (7) ppm. CH groups= (3.75) ppm.
2		OH phenolic groups =(7.30) pmm phenyl groups singlet at= (7.16) ppm. NH2 group = (3.02) ppm CH groups = (2.56) ppm
3		OH phenolic groups =(7.97) pmm NH amide group = (9.03) ppm CH groups = (2.75) ppm
4		OH phenolic groups =(7.65) pmm NH_2 group = (5.78) ppm, NH amide group =(7.16) ppm phenyl groups singlet at= (7.33) ppm. CH groups = (2.91) ppm
5		OH phenolic groups =(8.34) pmm phenyl groups singlet at= (7.72) ppm. NH group = (10.43) ppm CH groups = (3.34) ppm

* Compound 1 = Graphene oxide, Compound 2= GO-1-amino-2-naphthol-4sulfonic acid, Compound 3= GO-2-aminopyrimidine, Compound 4= GO-sulfamide, Compound 5= GO-dithiazone

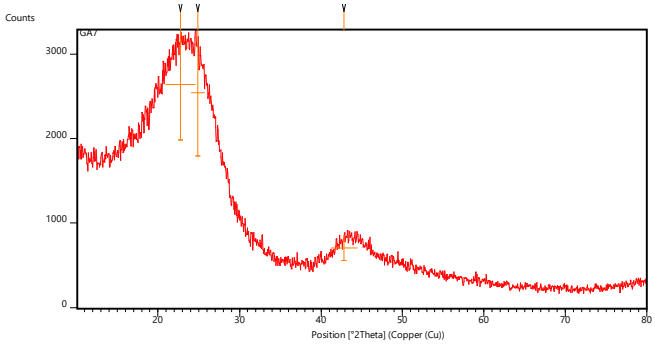


Figure 6: XRD of GO-1-amino-2-naphthol-4sulfonic acid

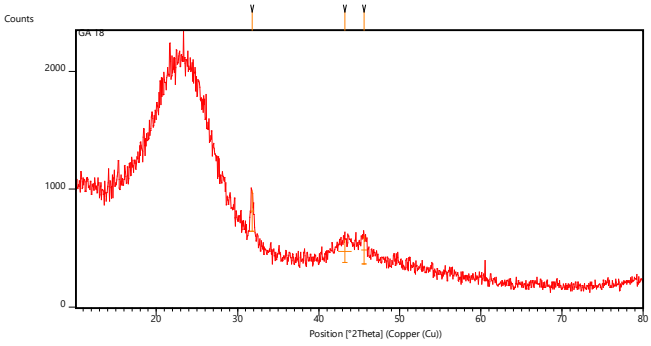


Figure 7: XRD of GO-sulfamide

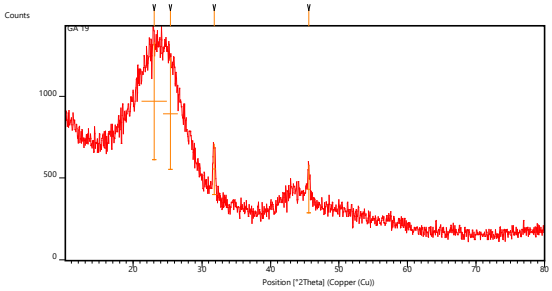


Figure 8: XRD of GO-dithiazone

carboxylic groups and converted graphene oxide to the reduced graphene oxide, which is functionalized graphene sheet. It was noticed that the difference was in the values of FWHM only, indicating the difference in particle size due to difference in substituted compound. Another observation among these three compounds is that compounds 4 and 5 showed a peak at locations 31.7969 and 31.7989 degrees, while it did not appear in compound 1, which means that this peak indicates the sulfone group in the substituted compounds (Table 3).

XRD of GO-1-amino-2-naphthol-4sulfonic acid, GO-sulfamide, GO-dithiazone

We notice in these compounds that no reduction of graphene oxide occurred by means of the added compounds, since

the amine group did not carry out the reaction as in the previous compounds (GO-1-amino-2-naphthol-4sulfonic acid, GO-sulfamide, GO-dithiazone). Therefore the results were like those obtained with compound (GO-aminopyrimidine). Furthermore, it should be noted that the diffraction pattern did not show the peak of graphene oxide, which gives evidence of the correctness of the reaction.[37] Moreover, it was also observed that these compounds had more crystalline characteristics than their predecessors (Table 3).

The Scherer equation was used to calculate the average particle size based on all the peaks that appeared in the XRD pattern, and the results were as follows: 3.54, 32.16, 10.1, and 11.45 nm for the compounds GO-1-amino-2-naphthol-4-sulfonic acid), GO-aminopyrimidine, GO-sulfamide and GO-dithiazone, respectively. The results of the calculations proved that the calculated minute size of the compounds resulting from the interaction of the amine group with graphene oxide was much less than the other two compounds, and this proves the occurrence of a reduction of graphene oxide to give amine-functionalized graphene sheets that are linked together by hydrogen bonds.[38] However, the particle sizes of these materials were not much in agreement with the obtained SEM measurement. This is due to the limited use of the Scherrer equation in spherical particles.

SEM Measurements

The SEM measurement of compound (GO-1-amino-2-naphthol-4sulfonic acid) revealed an irregular geometry, but it resembled nanoscale spheres with sizes ranging from 19.94-48.49 nm Figure 10A. The presence of this roughness in the measured surface is evidence of the success of the reaction of adding 2-aminopyridine to the he exterior walls of the graphene oxide sheet, since the reaction took place between each of carboxyl functional groups in graphene oxide and the amine groups in 2-amino pyrimidine. Roughness of the surface was evidence of the success of the reaction, since the surface of graphene oxide is mostly soft in SEM measurement. In Figure 10B, the measurement shows a laminar structure with a thickness of no more than 10 nm.[39]

The SEM measurement of (GO-2-amino pyrmidine) showed the presence of sheet-like nanostructures that were irregular and had an almost rough surface as in Figure 11A. In Figure 11b, it was found that the particle size on the surface was in the range 22.41–43.11 nm. As expected, the particle size here was almost similar to that obtained in (GO-1-amino-2-naphthol-4sulfonic acid), and this is that both of the two added molecules possessed one aromatic ring.[40]

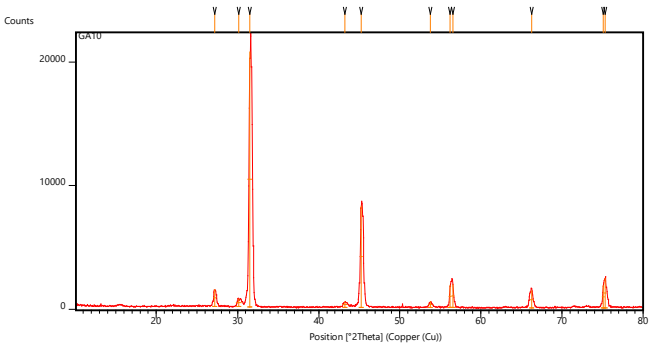


Figure 9: XRD of (GO-2-amino pyrmidine)

Table 3: XRD data and D average of the prepared nanocompounds

Compound	*Pos. [°2Th.]*	*FWHM Left [°2Th.]*	*d-spacing [Å]*	*Particle size (nm)*	*Average D (nm)*
GO-1-amino-2-naphthol-4sulfonic acid	22.7351	3.5424	3.91136	2.39	3.54
	24.8674	1.5744	3.58060	5.40	
	42.8817	3.1488	2.10903	2.83	
GO-2-amino pyrmidine	27.1662	0.2460	3.28260	34.71	32.16
	31.5190	0.4428	2.83850	19.48	
	45.2630	0.2460	2.00346	36.56	
	53.8063	0.3936	1.70379	23.65	
	56.5532	0.2460	1.62738	38.32	
	66.2971	0.3444	1.40988	28.79	
	75.0745	0.2400	1.26428	43.62	
GO-sulfamide	22.8241	3.5554	3.7772	2.38	10.1
	24.9153	1.6124	3.4900	5.27	
	31.7969	0.3936	2.81432	21.93	
	43.2487	1.5744	2.09198	5.67	
	45.6493	0.5904	1.98740	15.25	
GO-dithiazone	23.0434	3.5424	3.85972	2.39	11.45
	25.4229	1.9680	3.50360	4.32	
	31.7989	0.3936	2.81415	21.93	
	43.3356	1.5532	2.1000	5.75	
	45.6308	0.3936	1.98817	22.88	

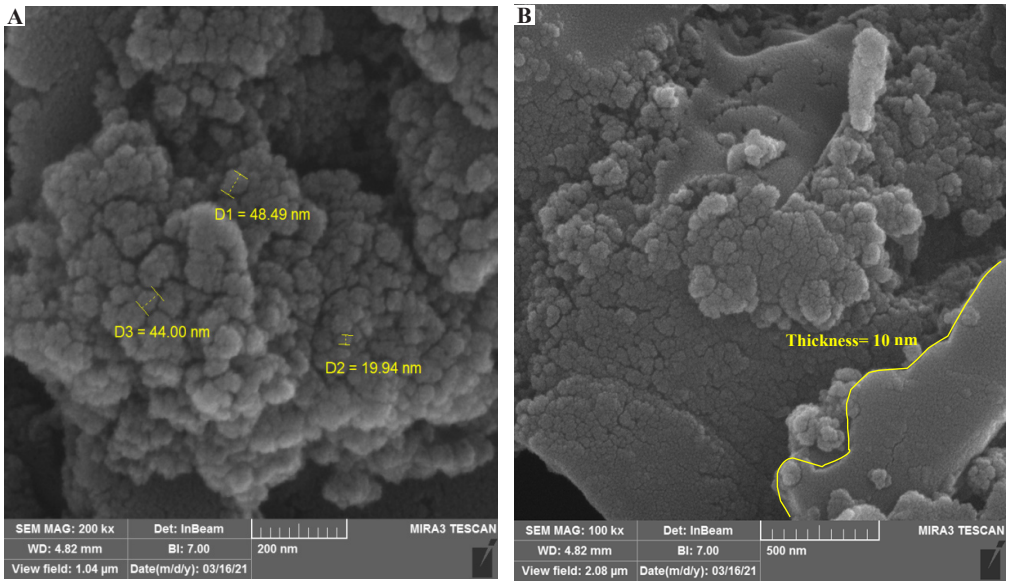


Figure 10: SEM image of compound (GO-1-amino-2-naphthol-4sulfonic acid)

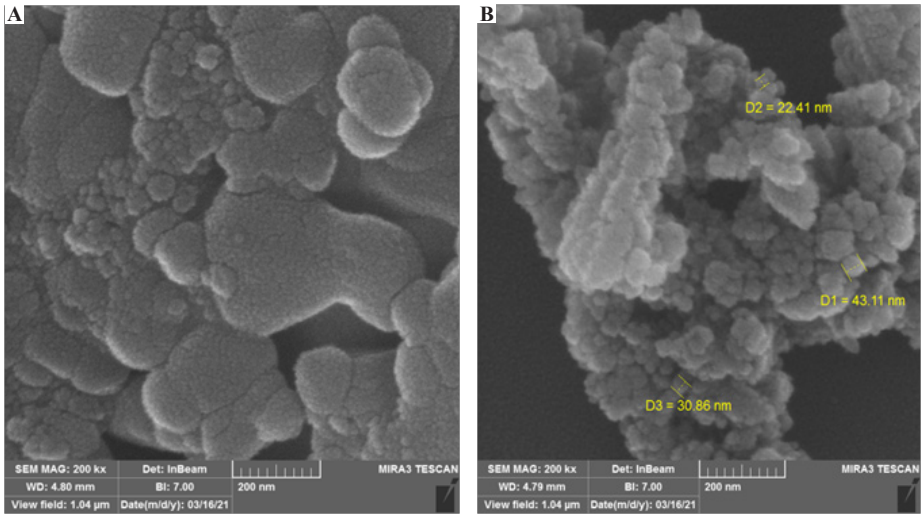


Figure 11: SEM image of (GO-2-amino pyrmidine)

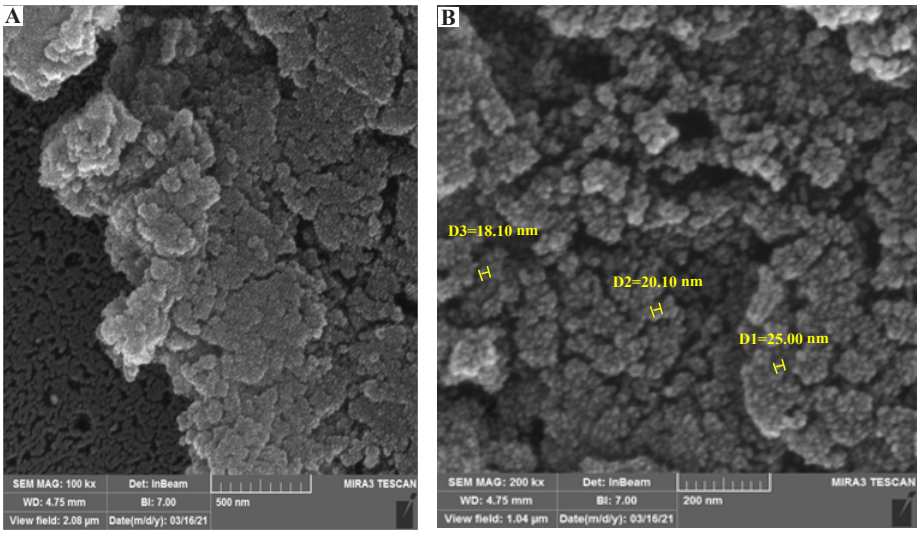


Figure 12: SEM image of (GO-sulfamide)

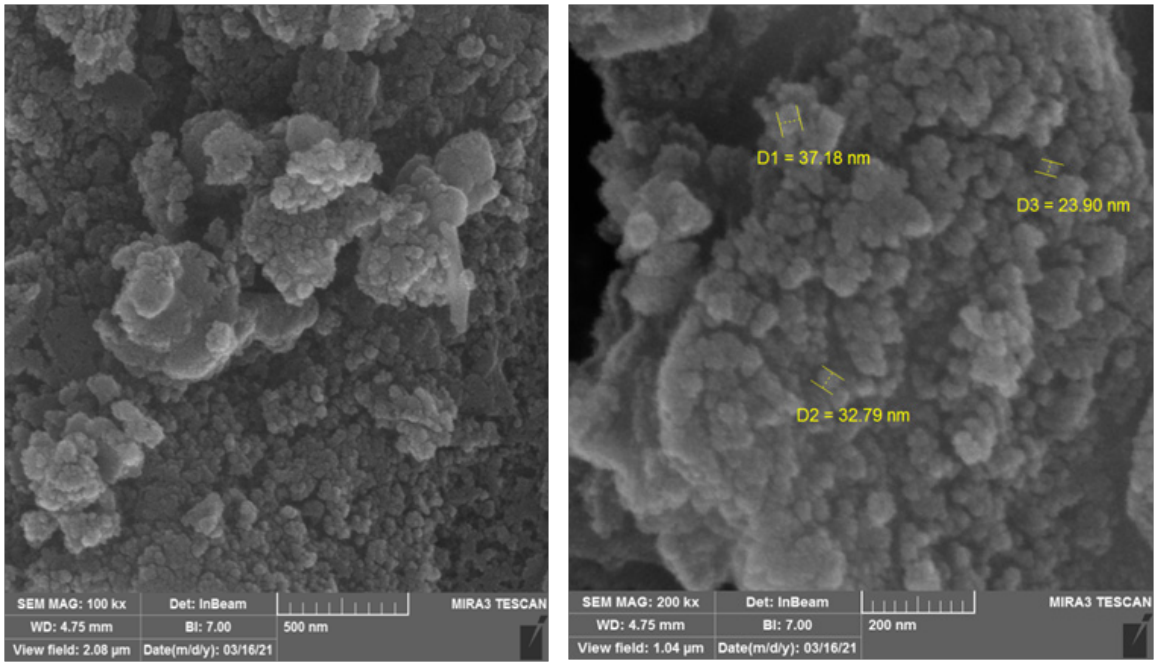


Figure 13: SEM image of (GO-Diathiazone

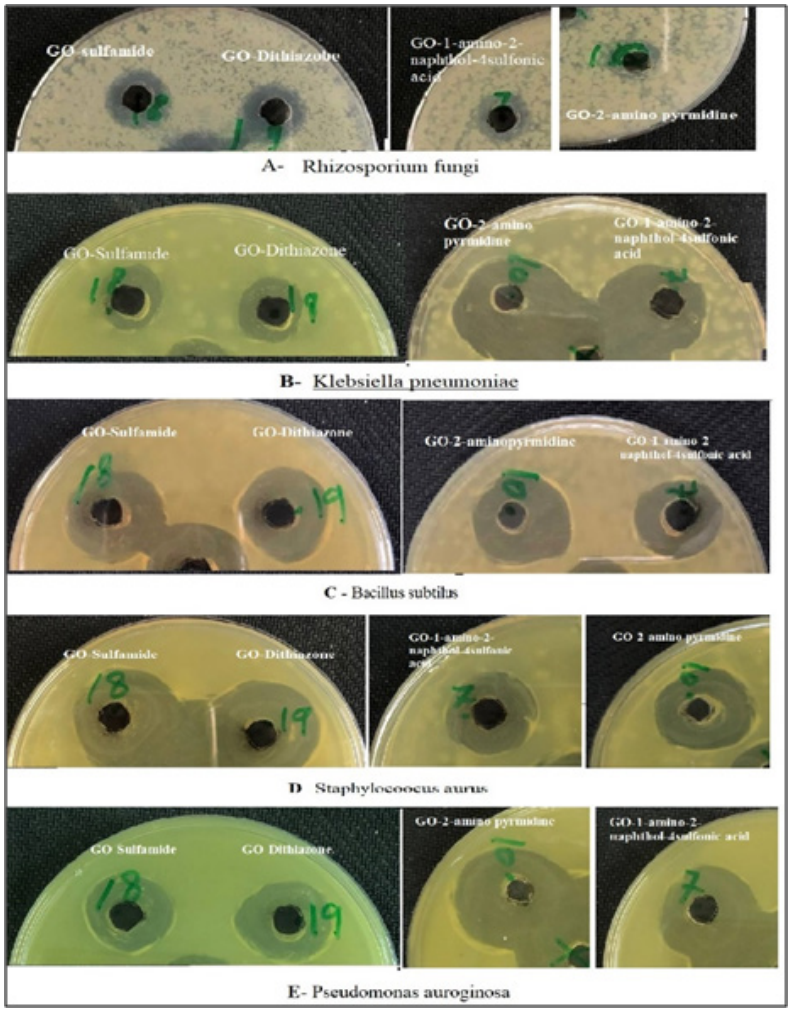


Figure 14: Antibacterial and antifungal activity.

The FESEM measurement showed of (GO-sulfamide) the presence of irregular sheet-like nanostructures as very close clusters with a relatively rough surface, as shown in Figure 12A. This assembly of sheets is evidence of the success of the reaction. It is noticeable that there was a much greater aggregation here than the previous compounds, and this can be attributed to the fact that the added molecule has two amine terminates capable of reacting and attaching to the graphene oxide sheets within its amino terminated group. In Figure 12B, the particle size at the surface was found to be in the range of 18.10–25.00 nm. The small size of the particles here is mainly because the additive molecule is a linear, small-sized molecule.[41].

The SEM measurement of (GO-Diathiazone) revealed an irregular nanostructure, but it resembled nanoscale spheres with sizes ranging from 23.90–37.18 nm, as in Figure 13. The presence of this roughness in the measured surface is evidence of the success of the reaction.

Antibacterial and Antifungal Activity

The measurement of antibacterial and antifungal activity was performed using the well-diffusion process. The development of an inhibition zone defined the sample's antibacterial activity. The Zone of Inhibition is the region on an agar plate from which an antibiotic, normally applied on the agar surface, inhibits a control organism's spread. If the test organism is resistant to the antibiotic, it would not have been able to establish in the presence of the antibiotic. The clear area around the antibiotic indicates its effectiveness; the larger the clear area around the antibiotic, the more influential the compound. For 24 hours, the sample's behavior was determined by the establishment of a Zone of Inhibition. Table 3 below shows the size of inhibition zone of graphene oxide derivative measured by (mm) with hole dimeter size 6 mm. the result of inhibition zones for compound 2 in gram/positive gram/negative and fungi (30, 22, 28, 22, 30, and 14 mm), respectively, the values for compound 3 shows (29, 19, 27, 19, 29 and 14 mm), also compound 4 shows resistance fore bacteria an fungi (19, 29,16, 22, 19, and 17 mm). However, compound 5 shows these values (20, 29, 16, 21, 20, 17 mm). The Figure 14 below shows the petri dishes for graphene oxide derivatives.

CONCLUSION

With Hummer method Graphene oxide and was synthesized then we reacted graphene oxide with (2-aminopyrmidine, Dithiazone, Sulfamide, and 1-amino-2-naphthol-4sulfonic acid) to get new derivative of graphene oxide, characterization of graphene oxide and its derivatives utilizing FTIR, H^1NMR, and XRD. The XRD patterns conform to its graphene oxide structure as hexagonal and the particle size was estimated using the (Debye-Scherer) equation and the (Williamson-Hall) equation.Since the average particle size using the (Debye-Scherer) equation is high, and (Williamson-Hall) to the ascribed width peaks to particle size and Internal emotion is modest when applying powders.Furthermore, FESEM studies of graphene oxide and its derivatives reveal that dark grey areas indicate numerous layers of sheets, whereas light grey areas indicate just a few layers. We use two gram +ve, two Gram -ve and one fungi for antimicrobial tests that show inhibition zones for all compounds, which means the effeteness of compounds on bacteria but less effeteness at fungi.

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