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Antimicrobial Activity Of Graphene Oxide (GO) And Reduced Graphene Oxide Nanocomposite Via Modified Hummer's Method.

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Abstract:
Materials possessing antimicrobial properties are of great interest for medicine. However, the safety of the modern materials on ammonium compounds, silver, ions, and barium sulphate standards. Graphene oxide is a promising candidate for biomedical research because itexhibits good antimicrobial properties with minimal cytotoxicity to human cells.The paper presents the results of studies of the antimicrobial activity of graphene oxide. The antimicrobial activity of the synthesized sample in screening one gram – negative bacteria and one gram-positive bacteria and one fungai was analyzed by using Kirby-Bauer method.The inhibition zones measurement of pseudomonas aerogenisa and Staph aureus due to the samples GO , RGO and Amikacin are tabulated.

Key words: Antimicrobial activity, Graphene Oxide, Reduced Graphene Oxide, Kirby-Bauer method, Pseudomonas aerogenisa, Staph aureus and Candida albicans.

1.Indroduction:
In recent year many scientific research has led to the discovery of the new materials, for example graphene oxide and reduced graphene oxide which has unique properties, inducing antimicrobial properties. GO consist is sp2-hybridized carbon atoms with oxygen containing
groups on their surface (hydroxyl, carboxyl, epoxy, etc.). GO synthesized by chemical decomposition; represent a suspension with individual monolayer flakes of graphene oxide. From this suspension obtained the graphene oxide film or graphene oxide paper by removing the solvent. Graphene paper is already used to create protective layers, chemical filters, components of electric batteries and supercapacitors, electronic and optoelectronic components, antibacterial medical bandages. All new possibilities of applications from graphene oxide paper are opened - flexible biosensors, biodegradable nanocomposites, and graphene inks. Graphene paper is an area of research promising qualitative changes in electronics, mechanics, optics, electrical engineering, medicine, and other fields. At the moment, much attention is paid to the study of nanostructures of GO as antimicrobial agents.[1,2,3] GO is asimilar 2D carbon sheet containing covalently bonded hydroxyl, epoxy, and carboxyl groups across the material basal plane.Owing to these additional moieties, 2D graphene oxide has been readily exploited as an active antimicrobial agent,[4,5] both in its native form and as a composite material.[7,8] Largely, studies of antibacterial GO composite materials have focused on the incorporation of silver nanoparticles, which also demonstrate significant levels of antibacterial activity. In the present study, we have reported the antibacterial activity of GO-RGO composite against the gram-negative bacteria -Pseudomonas aeruginosa and gram-positive bacteria – Staphylococcus aureus .The results were compared to a commercial antibiotic namely, amikacin. The antifungal activity of GO-RGO

composite against Candida albicans were also studied. The results were compared to a commercial antifungal medication namely, nystatin.

2.Bacterial growth: Kirby-Bauer method:

The antibacterial activity of GO and RGO nanocomposites was determined by Kirby-Bauer method. The medium is prepared and sterilized as directed by the manufacturer. . Defibrinated blood may be necessary for tests on fastidious organisms, in which case the medium should be allowed to cool to 50°C before 7% of blood is added. Human blood is not recommended as it may contain antimicrobial substances. The medium should be poured into petri dishes on a flat horizontal surface to a depth of 4mm 25ml in an 85 mm circular dish; 60ml in a 135mm circular dish. Poured plates are stored +4°C and used within one week of preparation. Before inoculation plates should be dried with lids ajar so that there are no droplets of moisture on the agar surface. The time to achieve this depends on the drying conditions. The pH of the medium should be checked at the time of preparation and should be 7.2 to 7.4. At least four morphologically similar colonies from an agar medium are touched with a wire loop and the growth is transferred to a test tube containing 1.5 ml of sterile suitable broth. The tubes are incubated for 2 hours at 35°C to 37°C to produce a bacterial suspension of moderate turbidity. The diameter of the inhibition zones was measured and reported. The reference antibiotic used in the study is amikacin. A similar procedure was adopted for the analysis of the antifungal activity of the synthesized GO-RGO composites. The reference antifungal used in the study was nystatin.

3.Materials and Methods

Chemicals and materials

Graphite flakes(Natural), Sodium Nitrate($NaNO_3$),Potassium Permanganate $KMnO_4$,DI water, and Hydrogen Peroxide (H_2O_2) with purity of 99.8% was purchased from sigma Aldrich. All Chemicals were of analytical grade and used as received.

3.1Synthesis of Graphene Oxide (GO):

Graphene oxide (GO) was prepared according to the modified Hummers method. In present details, 6g of graphite flakes and 3 g of $NaNO_3$ were mixed with 50 ml H_2SO_4 and stirred in an ice bath for 2hrs. Next 6g of $KMnO_4$ were slowly added so that the temperature of the mixture lower than below 5°C. The suspension was then reacted for 2 hrs in an ice bath and stirred for 1hr.The ice bath was then removed and mixture was stirred at 35°C until become the pasty brownish color and kept under stirring for 3 days. It is then diluted with drop by drop addition of 100 ml water. The reaction temperature was rapidly increased to 98°C with effervescence, and the color changed to brown color. Further the Deionized water was further added so that the volume of the suspension was 200ml. The final solution treated with 15ml 0f H_2O_2 was added after 5 minutes to terminate the reaction by appeared the yellow color solution. The reaction product was washed by rinsing and centrifuged and washed with deionized water and 5% Hcl solution repeatedly. After filteration and drying under vaccum at room temperature. The final product was dried at 60°C at using muffle furnace. The graphene oxide (GO) was obtaine as a powder.

3.2 Synthesis of Reduced Graphene Oxide:

For the reduction of Graphene oxide using Hummer's method, One of the Most commonly used reducing agent is hydrazine Hydrate.[9] One major reason for its popularity is its inertness to water, Which is present in GO as a dispersing solvent. Nevertheless, after careful studies of the products of the reduction process coupled with the knowledge of the reaction mechanisms of hydrazine with other organic species, it is suggested that the reduction of GO is similar to that reaction with hydrazine.[10]The technique consists of the initial oxidation of graphite to graphite oxide, followed by the subsequent chemical exfoliation of graphite oxide to graphene oxide.[11,12,13,14]

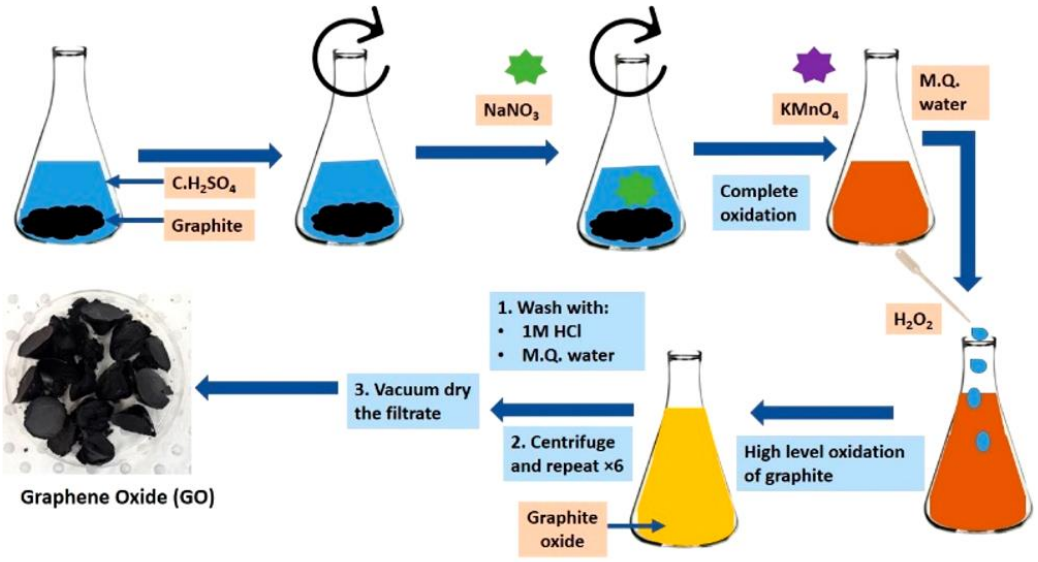

Figure 1. Schematic representation of synthesizing GO.

3.3 Materials Characterization:

x-ray diffraction (X'PERTPOWDER x-ray diffractometer) were used to characterize the as-prepared samples. For visualization combination of Scanning Electron Microscopy(SEM) and Chemical composition analysis of Energy Dispersive Spectrum (EDAX).

4.Results and discussion:

4.1 Structural Analysis:

X-ray diffraction is one of the resolvable characterization to observe the crystallinity of the GO. The XRD was performed using XPERT-PRO diffractrometer with CuKα radiation at wavelength 1.5406A°. The Scanning angle was done in the range from 2θ= 10° to 80°. The X-ray Diffractration patterns for graphite powder, graphen oxide powder and reduced graphene oxide powder were recorded. The average of crystallite size (D) of the GO-RGO was calculated from Scherrer's equation for the (002) plane.[15]

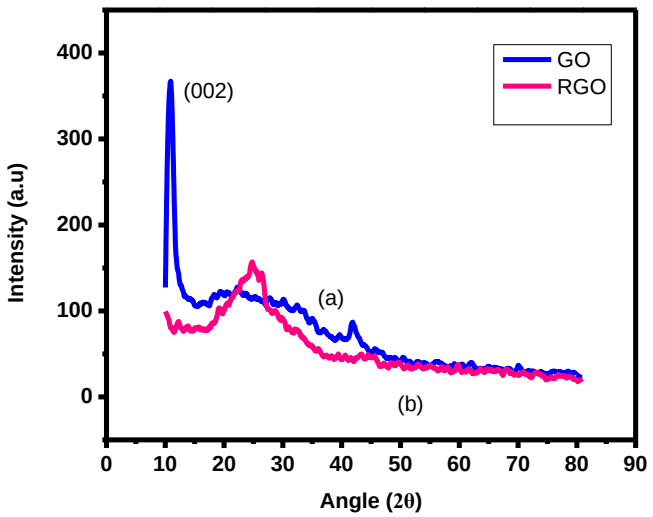

Figure 2. XRD pattern of GO and RGO nanoparticles.

4.2 Morphological Analysis:

The microstructure of the prepared materials was characterized by Scanning Electron Microscopy (SEM) analysis. The SEM micrographs of graphene oxide nanoparticles show in Fig (3) . From the figure, it can be observed that graphene oxide has layered structure, which affords ultrathin and homogeneous graphene

oxide powders.The figure shows the a thickened nanostructure with rough and from the SEM micrographs of GO nanoparticles of average diameter Value 2.02nm.The SEM image also confirm that functionalized on the surface of the graphene oxide nanoparticles was identified by an energy-dispersive x-ray spectroscopy (EDX) study. The EDS spectrum of GO nanoparticles show in figure (4) confirmed the presence of carbon (52.33 %) and Oxygen (47.67%) in the Table. The results of the EDX spectrum also confirm the successful formation of GO nanoparticles.

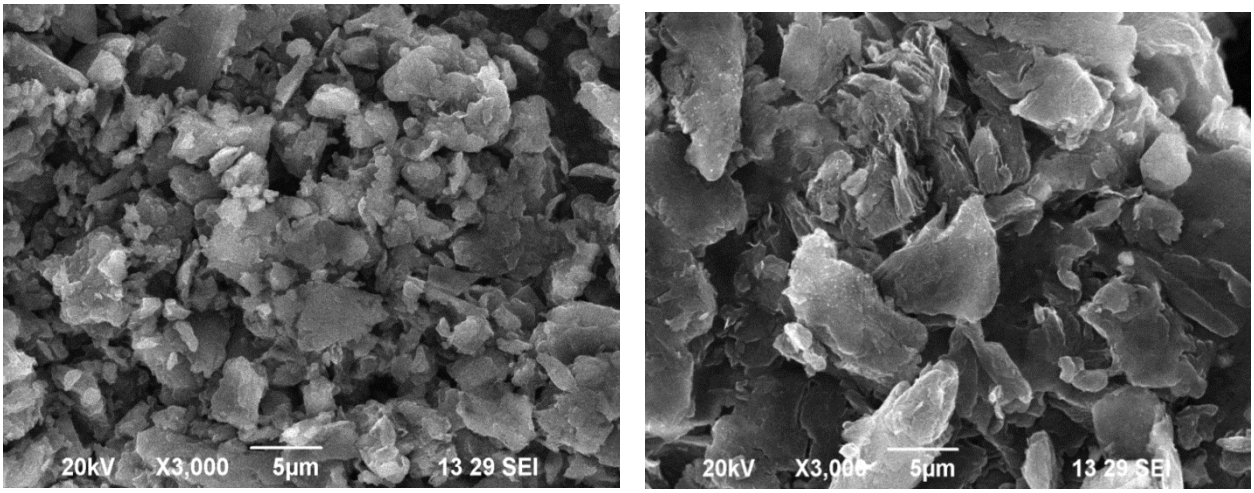

Figure 3. SEM image of GO and RGO nanoparticles

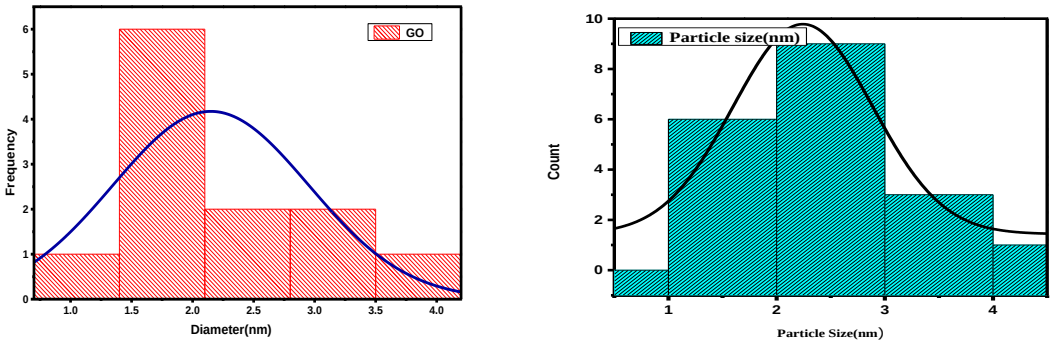

Figure 4.SEM image of GO and RGO NPs along with particle size histogram.

Table 1 :The elemental composition of Graphene Oxide Nanoparticles in EDAX spectrum

Element	Weight %	Atomic %
C K	45.18	52.33
O K	54.82	47.67

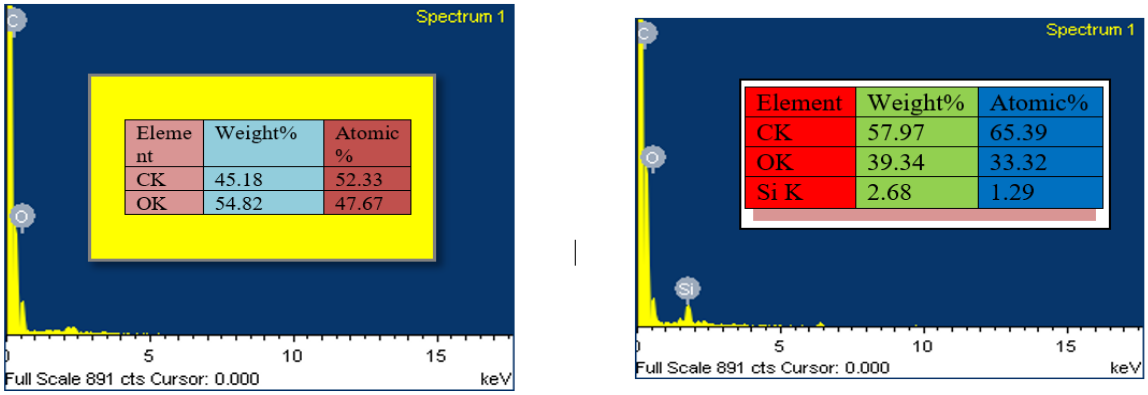

Figure 5. EDAX spectrum of GO and RGO NPs

Table 2 :The elemental composition of Reduced Graphene Oxide Nanoparticles in EDX spectrum

Element	Weight %	Atomic %
C K	57.97	65.39
O K	39.34	33.32
Si K	2.68	1.29

4.3 Antibacterial Activity:

The antibacterial activity of Graphene oxide (GO) and Reduced Graphene oxide (RGO) nanocomposite was carried out two bacterial systems: Pseudomonas aerogenisa (Gram-negative)
And Staph aureus (Gram-positive). The antibacterial activity of GO and RGO NCs tested against bacterial system as shown in figure (6). The inhibition zones measurement of Pseudomonas aerogenisa and staph aureus due to the samples GO , RGO and Amikacin are tabulated in table (3) and pictorially represented in figure(6). It is observed that the synthesized samples show greater efficiency in screening Pseudomonas aerogenisa to Amikacin. It is also noticed that GO and RGO decrease with increase of composition.

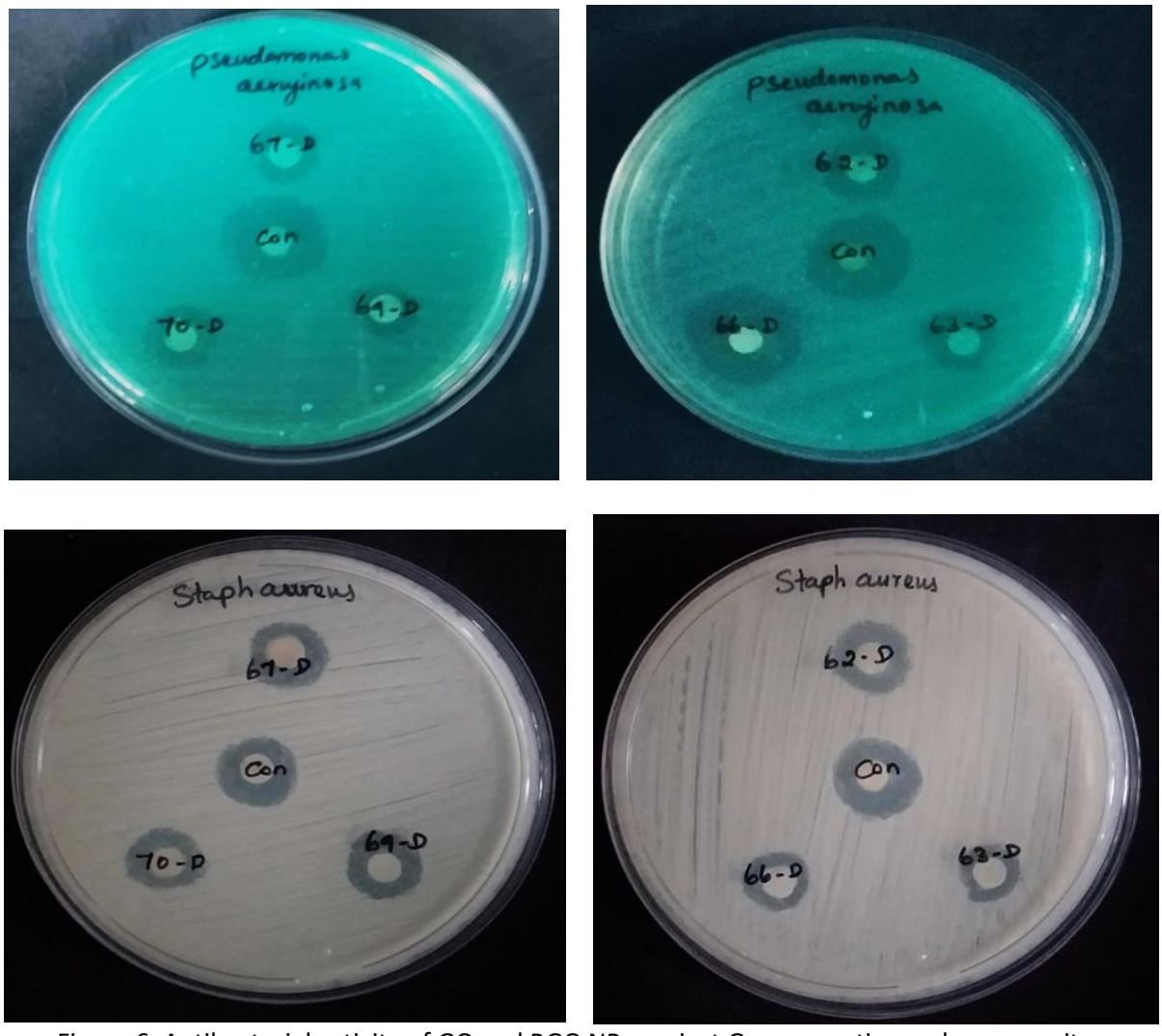

Figure 6. Antibactorial activity of GO and RGO NPs against Gram-negative and gram-positve

Table 3: Antibacterial activity of GO and RGO NPs against Pseudomonas aeruginosa and Staph aureus obtained by Kirby –Bauer method.

S.No	Samples	Antibacterial Activity (Gram Negative bacteria)

		Pseudomonas aeruginosa	Staph. aureus
1	Amikacin	20mm	16mm
2	Graphene Oxide (GO)	13mm	15mm
3	Reduced Graphene Oxide (RGO)	22mm	12mm

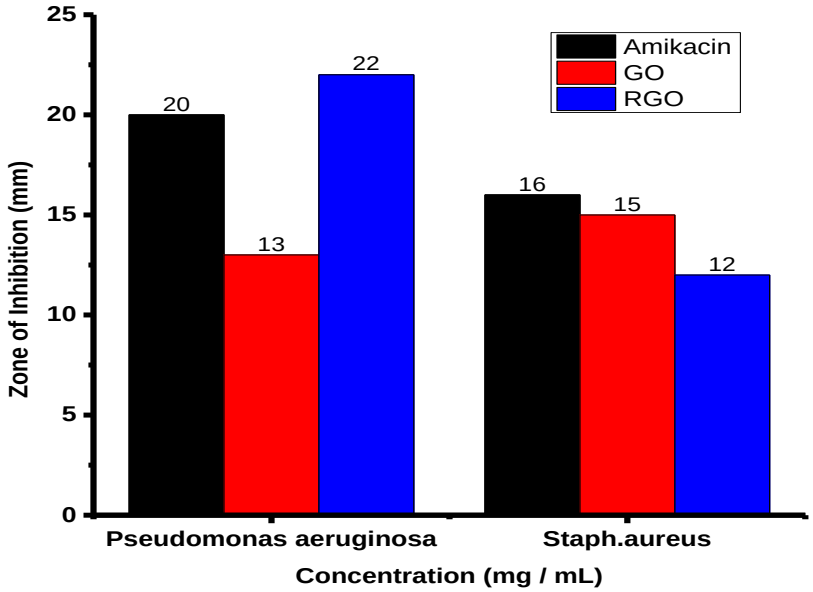

Figure 7.Hiatogram showing the Zone inhibition of Go and RGO gram –negative and gram-positive bacteria

4.4 Antifungal activity:

The inhibition zones measurement of Candida albicans due to the samples GO, RGO and nystatin are tabulated in table (4) and pictorially represented in fig.(8). The synthesized samples show good antifungal activity against both the fungi. The antifungal activity of is observed to decrease with an increase in the GO and RGO NPs composition of the composite. However, the diameter of inhibition zones produced by both GO and RGO is greater compared to that of nystatin.

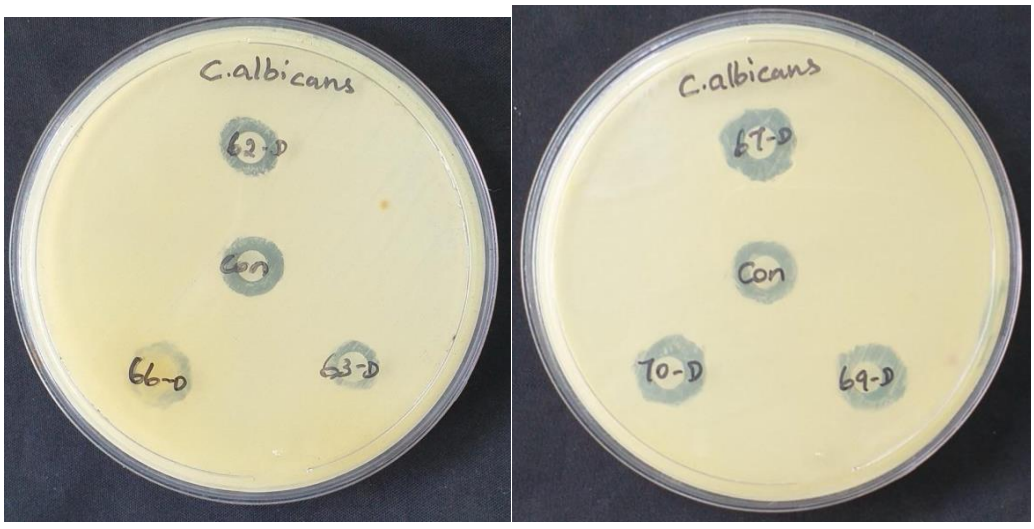

Figure 8. Antifungal activity of GO and RGO NP

Table 4: Antifungal activity of GO and RGO NPs against Candida*. albicans* obtained by Kirby-Buaer method.

S.NO	Samples	Antifungal Activity
		Candida .albicans
1	Nystatin	12mm
2	Graphene Oxide (GO)	15mm
3	Reduced Graphene Oxide (RGO)	14mm

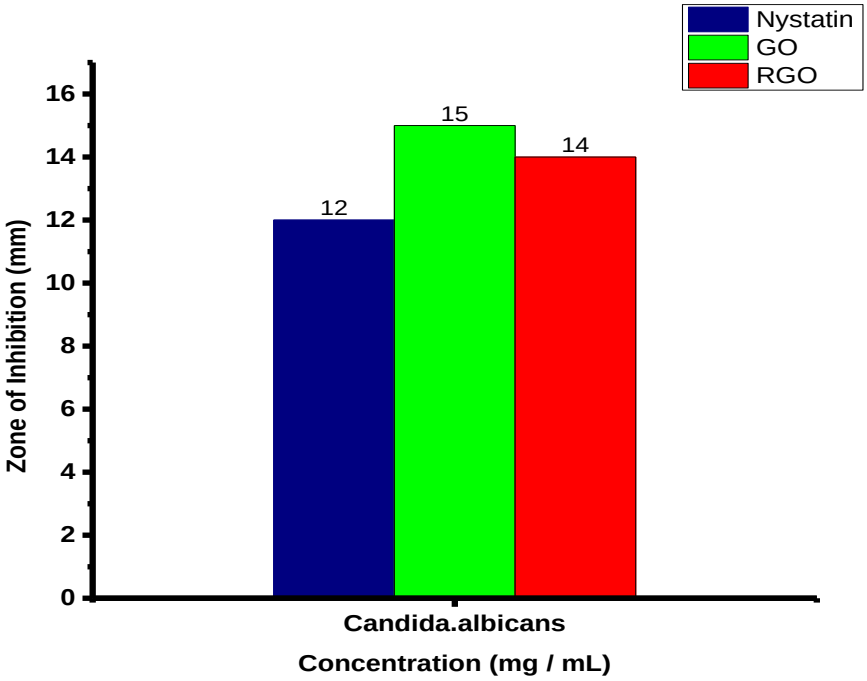

Figure 9.Hiatogram showing the Zone inhibition of Go and RGO of Antifungal activity.

5. Conculsion:

GO and RGO hybrid compounds were successfully produced and characterized**.**The Antimicrobial Activity of synthesized Graphene Oxide (GO) and Reduced Graphene Oxide (RGO) was studied against Pseudomonas aerogenisa, Staph aureus and Candida albicans using Kirby-Bauer Method. Thus the obtained results indicate a pronounced antimicrobial activity of 13mg /ml and 15mg/ml for Pseudomonas aerogenisa gram-negative and Staph aureus gram-positive bacteria. GO sensitivity is found in bacteria and fungai. Thus, the obtained results prove the promising application of GO as an inexpensive and highly effective antimicrobial carbon nanomaterial.

Authors Contributions:

Thangaraj.N: First Author- Conceptualization (lead); data curation (lead); investigation (lead); methodology (lead); writing – original draft (lead); writing – review and editing(lead).

Joseph John.N: Conceptualization (lead); data curation (lead); investigation (lead); methodology (lead); supervision (lead); writing – original draft (lead);writing – review and editing (lead).

Gnana Sambandam.C : Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); validation (equal); writing – original draft(equal); writing – review and editing (equal).

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Conflict of interest statement :
The authors declare no conflict of interest.

Data availability statement:
Data shall be made available upon reasonable request.

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