Antibacterial Activity Study of Graphene Oxide-Polyaniline (GO-PANI) and GO-PANI-Mupirocin Composite against *Escherichia coli* and *Staphylococcus aureus*

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Abstract— Graphene Oxide (GO) and Polyaniline (PANI) were synthesized by modified Hummers method and Oxidative polymerization respectively. GO-PANI and GO-PANI-Mupirocin composites were synthesized by simple dispersion methods during polymerization. The synthesized composites were characterized by Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FT-IR). Characterization results confirmed their formation. Antibacterial activity of prepared composites was tested by well diffusion method for three different concentrations, 100µg/mL, 200µg/mL and 300µg/mL against two prokaryotic bacterial species, S. aureus and E. coli. Bacterial growth was inhibited by the composites sample and measured by zone of inhibition (ZOI) diameter. Both composites showed concentration dependent activity i.e. activity increased with increasing concentration. The minimum ZOI diameter was 13.5 mm and the maximum was 27.0 mm means that the synthesized composites had greater antibacterial activity against the bacteria. As an antibiotic, Mupirocin was used, GO-PANI-Mupirocin composite showed greater activity than GO-PANI. Since these composites inhibited the bacterial growth which indicates that they may act as anti-microbial agent for different microorganisms.

Index Terms— Antibacterial Activity, Composite, Graphene Oxide, Mupirocin, Polyaniline, Well Diffusion, Zone of Inhibition

1 Introduction

THE increasing resistance of the microorganisms towards antibiotics has been led to serious health problems in the recent years. Most infection-causing bacteria are resistant to at least one of the antibiotics that are generally used to eradicate the infection. This problem encourages the researchers to study the new agents which can effectively inhibit microbial growth [1].

Graphene oxide (GO) have recently emerged as a new allotropic form of carbon that provides an alternative path to Graphene [2]. It is a single-atomic-layered material which is obtained by the synthesis of graphite with strong oxidizing agents, available in large quantities at inexpensive prices. Structurally GO is chemically modified Graphene, containing hydroxyl, carbonyl and epoxy functional groups [3]. Since these groups have a high affinity to water molecules, it is hydrophilic and can be easily dissolved in water and other solvents allows it to be uniformly deposited onto wide ranging substrates in the form of thin films or networks, which makes it potentially useful for microelectronics, such as field effect transistors, solar cells, sensors and adsorbent for heavy metal removal [4], [5]. It has been also used as a promising material for preparing new composites [6]. It is well known that GO and its composites possess anti-microbial properties and have been used as anti-bacterial and antifungal agents [7], [8]. GO is reported possess antimicrobial property against various microbial. There are three steps in the mechanism of antibacterial activity of GO towards *Escherichia coli* [9]. Inhibition activity also shown by GO against *Escherichia coli* and *Pseudomonas aeruginosa* [10].

Conducting polymers have attracted a plethora of interest among scientists and researchers in recent years owing to its potential applications in various fields like electrochemical display, sensors, catalysis, redox capacitors, electromagnetic shielding and in secondary batteries. Among the conducting polymers, Polyaniline (PANI) is one of the promising polymers due to high conductivity, simple synthesis procedure, good environmental stability and reversible acid base chemistry in aqueous solution and large variety of applications. PANI exists in a variety of forms that differ in chemical and physical properties. After the polymerization from aniline monomer, PANI can be found in one of the three idealized oxidation states; Leucoemeraldine-white/clear and colorless fully reduced state, Emeraldine-green for emaraldine salt (ES) whereas blue for emaraldine base (EB) and Pernigraniline-blue/violet fully oxidized state [11].

When our skin comes to a wide range of bacterial invaders, the skin is able to present a first line defense against these invaders. However when the skin's natural defense weakens accidentally or intentionally, the role for antibacterial emerge. Most antibacterial used topically offer several advantages, such as avoidance of systemic toxicity leading to fewer side effects, decreased induction of bacterial resistance, and the

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high concentration of antibacterial agent at the site of infection [12]. One good example is mupirocin (pseudomonic acid A), an antibiotic produced by Pseudomonas fluoresces which has been shown to possess high activity against *staphylococci*, *streptococci*, and certain Gram-negative bacteria such as *Neisseria gonorrhea* and *Saemophilus influenza*. Its clinical use has been limited to topical preparations, commonly available as a 2% ointment, due to its high protein binding and marked reduction of its antibacterial activity in the presence of human serum [13]. Its mechanism of action is by inhibiting bacterial protein synthesis by specific reversible binding to bacterial isoleucyl tRNA synthase [14].

In the present study, we prepared GO-PANI and GO-PANI-Mupirocin composites. The prepared specimen was evaluated for the antibacterial activity test against Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* bacteria. Various methods were used to identify the structure and chemical nature of the composites. Though, identically both GO and PANI has antibacterial activity revealed by many researchers, but their composite's antibacterial activity still now unexposed. So we hope that, this information may be useful to evaluate the potential use of these composite incorporated with antimicrobial agents as a prophylactic use against bacterial infections in the near future.

2 METHODOLOGY

2.1 Chemicals

Flake graphite, Sodium nitrate, Potassium permanganate, Sulfuric acid (98%), Hydrogen peroxide, Aniline, Ammonium peroxodisulfate (APS), Cetyl trimethyl ammonium bromide (CTAB), Hydrochloric acid (35%) obtained from Merck, Germany with high purity and Mupirocin obtained from Sigma Aldrich with 99.5% purity. Deionized water (H_2O) was used as solvent to prepare all of the solutions utilized in this work.

2.2 Preparation

Graphene oxide (GO) and Polyaniline (PANI) were prepared by modified Hummers' method [15] and oxidative chemical polymerization [16] respectively. GO-PANI and GO-PANI-Mupirocin composite were prepared by the dispersion of GO and Mupirocin solution separately due to the polymerization of aniline.

2.2.1 Preparation of GO-PANI Composite

Aniline monomer (5 mL) was transferred into the 100 mL beaker containing 10 mL water and kept in ice bath with continuous stirring for 5 min. Then 35% HCl (5 mL) was added and stirred for 5 min. 500 mg Cetyl trimethyl ammonium bromide (CTAB) (500 mg) was transferred in 100 mL beaker containing 10 mL water under stirring for 5 min till it dissolved in water. Then 500 mg Ammonium peroxodisulfate (APS) was transferred in a 100 mL beaker containing 10 mL water under stirring for 5 min till it dissolved in water and then added to the monomer contents drop wise. After then solid GO powder (30 mg) was dissolved in 70 mL water and ultrasonicated for 30 min till it disperse in water and then added to the monomer contents drop wise and ultrasonicated for additional 2 hr. The contents were then washed several times with double distilled water. Finally, the mixture was centrifuged to obtain the GO-PANI solid composite which was then dried overnight in oven at 70^0C [17, 18].

2.2.2 Preparation of GO-PANI-Mupirocin Composite

Aniline monomer (5 mL) was transferred into the 100 mL beaker containing 10 mL water and kept in ice bath with continuous stirring for 5 min. Then 35% HCl (5 mL) was added and stirred for 5 min. 500 mg Cetyl trimethyl ammonium bromide (CTAB) was transferred in a 100 mL beaker containing 10 mL water under stirring for 5 min till it dissolved in water. Then 500 mg Ammonium peroxodisulfate (APS) was transferred in a 100 mL beaker containing 10 mL water under stirring for 5 min till it dissolved in water and then added to the monomer contents drop wise. After then solid GO powder (30 mg) was dissolved in 70 mL water and Mupirocin (20mg) was dissolved in 80 mL water separately and ultrasonicated for 30 min till dispersion in water and then added to the monomer contents drop wise and ultrasonicated for additional 2 hr. The contents were then washed several times with double distilled water. Finally, the mixture was centrifuged to obtain the GO-PANI solid composite which was then dried overnight in oven at 70^0C [17, 18].

2.3 Antimicrobial Activity Test by Well Diffusion Method

2.3.1 Bacterial strains

Clinically important two microorganisms, one prokaryotic Gram negative bacteria *Escherichia coli* (ATCC-25922) and other prokaryotic gram positive bacteria *Staphylococcus aureus* (ATCC 11632) were included in the study.

2.3.2 Culture of Bacteria

Bacteria were cultured on Mueller Hinton broth (MHB) for 24 hour. Four to five well isolated colonies were transferred and inoculated using sterile loop into the tube of sterilized 0.8% saline solutions (10 mL) having 3 ml of Mueller Hinton broth (MHB). The inoculums was emulsified inside the saline tube to avoid clumping of the cells and incubated at 37°C for three hours. The broth culture was adjusted to 0.5 McFarland standards which equal approximately $8x10^8$ CFU/mL of broth.

2.3.3 Preparation of Nutrient Agar Medium

To carry out the antibacterial activity test, Mueller Hinton Agar (MHA) medium as nutrient agar medium was prepared by using beef extract (2.0 g), acid hydrolysate of casein(17.5 g), starch (1.5 g) in 1000 mL distilled water and the pH was adjusted to 7.0 and agar (17.0 g) was added to the solution. The agar medium was sterilized in aquilots of 15 mL at a pressure of 15 lbs for 15 min. This nutrient agar medium was transferred into sterilized petri dishes in a laminar air flow unit and allowed to solidify.

2.3.4 Antibacterial Activity Test of GO-PANI and GO-PANI-Mupirocin Composite

Susceptibility of isolates to GO-PANI and GO-PANI-Mupirocin composites sample was measured in vitro by employing well diffusion method. This method allows for the rapid determination of the efficacy of the sample by measur-

ing the diameter of the zone of inhibition that results from diffusion of the sample reagent into the medium surrounding the well. After solidification of the MHA media, a 24 hours culture of each organism was standardized to 0.5 McFarland standard culture, which was then cultivated as a lawn culture on the solid surface of the MHA medium. A cotton swab was dipped into broth tube containing young culture of microorganism and was streaked evenly in three directions over the entire surface of the MHA plate for uniform inoculums to obtain confluent growth. The plate was then allowed to dry for 30 to 45 minutes. Wells were made into MHA. GO-PANI and GO-PANI-Mupirocin composites sample (100µg/mL, 200µg/mL and 300µg/mL) were then poured to the wells of the inoculated plates using micropipette within 5 min after the plates were placed in an incubator at 37°C. After 36 to 48 h of incubation, inhibition zone diameter around disc was measured in mm using Intech antibiotic zone reader (model IN - 1215, India). All the experiments were done in triplicate and average values were taken into account.

3 Results And Disscussion

3.1 Scanning Electron Microscopic (SEM) Analysis

The synthesized GO-PANI Composite and GO-PANI-Mupirocin composites were studied by SEM (JEOL, JSM-7600F, USA). A fixed working distance of 8.0-9.3 mm and a voltage of 5-10 kV were used.

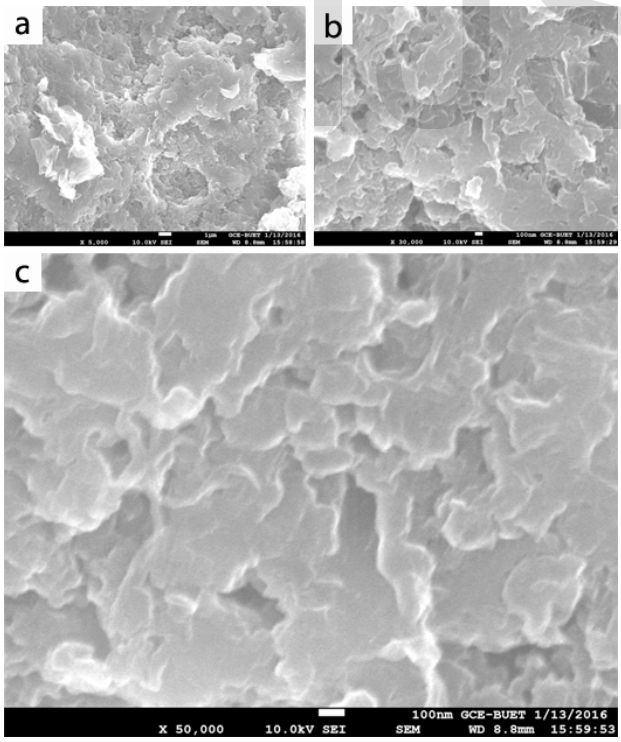

Fig. 1. SEM images of GO-PANI composite at the magnifications (a) 5000, (b) 30000 and (c) 50000.

A coralline-like morphology of GO-PANI composite was observed in which GO host was surrounded by PANI shown in Fig. 1. In addition, each flaky layer of the GO uniformly piles up while layers of composite have individual directions due to the influence of PANI, indicating that Oxidative polymerization affects the ordering structure of GO. Where PANI was randomly distributed around the GO sheets and a strong bonding between PANI and GO could be observed.

On the other hand the GO-PANI-Mupirocin composite also formed a coralline-like morphology which attributed that GO host was surrounded by PANI particles followed by Mupirocin shown in Fig. 2. Taking a closer view on the composite surface, it was surprised to observe the rough PANI coating layer on the GO sheet which is further coated by Mupirocin formed a sandwich like structure.

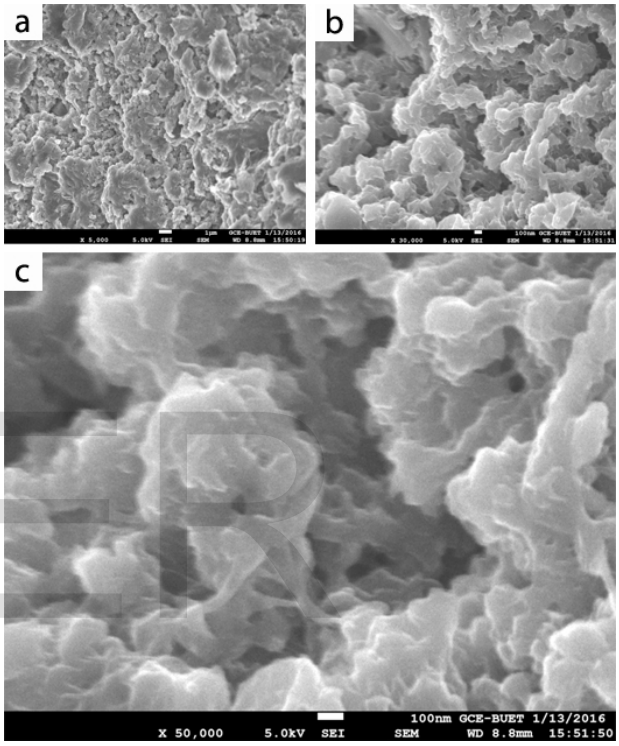

Fig. 2. SEM images of GO-PANI-Mupirocin composite at the magnifications (a) 5000, (b) 30000 and (c) 50000.

3.2 Fourier Transform Infrared Spectroscopic (FT-IR) Analysis

FT-IR (Model 3116465, Shimadzu, Japan) spectrum of GO-PANI composite was shown in Fig. 3. Some sharp peaks appeared at 1556 cm^{-1} indicated the presence of benzene ring. Several low intense peaks at 800 cm^{-1}, 820 cm^{-1} and 880 cm^{-1} indicated the out-of-plane deformation (bending) of =C-H for monosubstituted or 1,2-disubstituted ring and out-of-plan bending of N-H for amine, 1105 cm^{-1} were due to the stretching vibration of C-N bond, 1230 cm^{-1} and 1290 cm^{-1} respectively showed the presence of stretching C-N bond on primary and secondary amine, 1730 cm-1 due to C=O stretching of carboxylic acid group, 2922 cm^{-1} due to aromatic ring stretching of C-H bond, 2922 and 2852 cm^{-1} also for the O-H stretching of carboxylic acid group, a broad and wide peak at 3443 cm^{-1} attributed to the O-H stretching vibrations of the C-OH groups and for C-N stretching of primary and secondary amine.

FT-IR spectrum of GO-PANI-Mupirocin composite was shown in Fig. 4. There were characteristic peaks of GO and PANI as

usual shown in Fig. 3. Principal peaks for Mupirocin appeared at 950-764 cm^{-1} for epoxy group, 1352 cm^{-1} for C-O stretching of ether, 1645 cm^{-1} for conjugation of C=O with unsaturated C=C bond, 1713 cm^{-1} for C=O for ketone, carboxylic acid and ester group. At 3435 cm^{-1}, 2925 cm^{-1} and 2861 cm^{-1} appeared large wide and low intense peak for O-H stretching of carboxylic acid and C-OH group which meant that most of the OH and COOH group vanished through bonding.

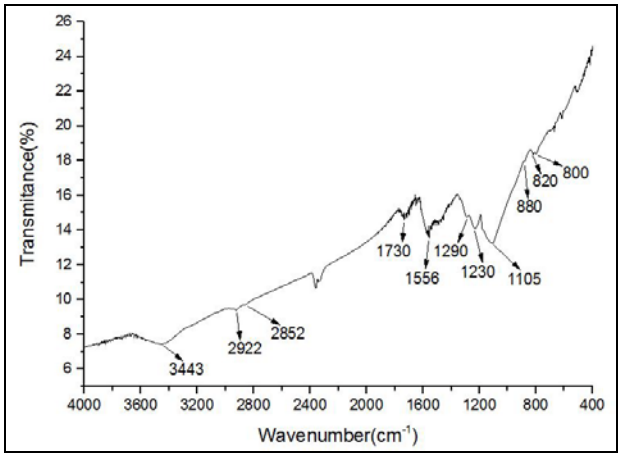

Fig. 3. FTIR analysis of GO-PANI composite.

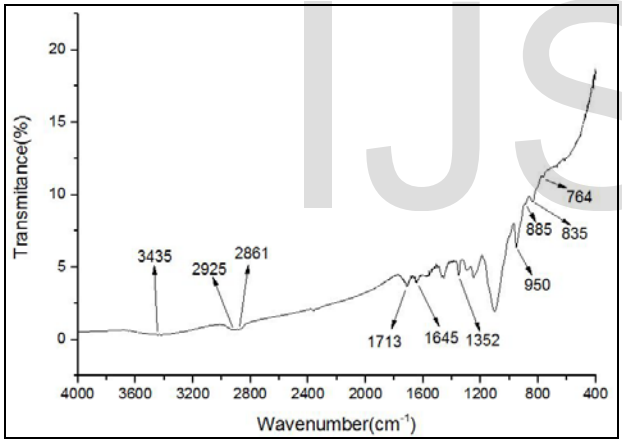

Fig. 4. FTIR analysis of GO-PANI-Mupirocin composite.

3.3 Antibacterial Activity Study

Consequence of antibacterial activity demonstrates the formation of zone of inhibition (ZOI). Zone of inhibition is the area on an agar plate where growth of a control organism is prevented by an antibiotic/antibacterial agent usually placed on the agar surface. If the test organism is susceptible to the antibiotic, it will not grow where the antibiotic is present. The size of the zone of inhibition is a measure of the compound's effectiveness, the larger the clear area around the antibiotic/antibacterial agent, the more effective the compound. The clear zone surrounding the samples in the remaining plates showed the activity of the samples. The zone surrounding the samples was clear that showed complete zone of inhibition. The space surrounding the complete zone of inhibition was partial zone of inhibition where the activity decreased than complete zone of inhibition. The results showed that the zone of inhibition increased with the increasing concentration of the samples on the entire microorganism.

3.3.1 Antibacterial Activity Study of GO-PANI Composite

The zone of inhibition of GO-PANI composite against *E. coli* and *S. aureus* were shown in Fig. 5. (a) and (b). As the concentration of GO-PANI composite increased, the zone of inhibition also increased. The inhibition zone diameter against *E. coli* were 17.0 mm, 22.0 mm, 24.5 mm and against *S. aureus* were 13.5 mm, 18.5 mm, 23.5 mm for the concentration 100μg/mL, 200μg/mL and 300μg/mL respectively.

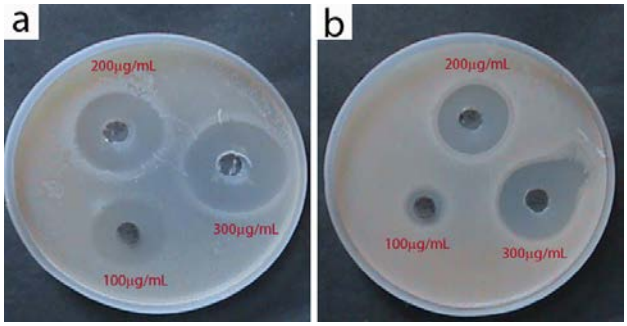

Fig. 5. Formation of Zone of inhibition against (a) *E. coli* and (b) *S. aureus* by GO-PANI composite.

3.3.2 Antibacterial Activity Study of GO-PANI-Mupirocin Composite

GO-PANI-Mupirocin composite showed considerable antibacterial activity on both the bacteria tested. Formation of zones of inhibition of GO-PANI-Mupirocin composite against *E. coli* and *S. aureus* were shown in Fig. 6. (a) and (b). The diameter of inhibition zone measured for *E. coli* were 18.0 mm, 26.5 mm, 27.0 mm and for *S. aureus* were 16.0 mm, 18.5 mm, 23.0 mm for the concentration 100 μg/mL, 200 μg/mL and 300μg/mL respectively.

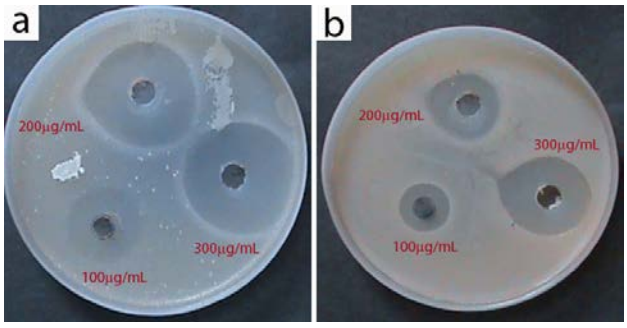

Fig. 6. Formation of Zone of inhibition against (a) *E. coli* and (b) *S. aureus* by GO-PANI-Mupirocin composite.

TABLE 1

Inhibition zone diameter of GO-PANI and GO-PANI-Mupirocin composite against *E. coli* and *S. aureus*

Synthesized Sample	Bacteria					
	E. coli			*S. aureus*		
Concentration (μg/mL)	100	200	300	100	200	300
	Diameter of inhibition zone (mm)					
GO-PANI composite	17.0	22.0	24.5	13.5	18.5	23.5
GO-PANI-Mupirocin composite	18.0	26.0	27.0	16.0	18.5	23.0

4 Conclusion

This study demonstrated the preparation of GO and PANI NPs by using modified Hummers method and oxidative chemical polymerization respectively. GO-PANI and GO-PANI-Mupirocin composites were synthesized by simple dispersion method during polymerization. Characterization was done by SEM and FTIR analysis. Characterization results confirmed the formation of the species.

Antibacterial activity test was done by agar diffusion method for three different concentrations of prepared GO-PANI and GO-PANI-Mupirocin composites as 100μg/mL, 200μg/mL and 300μg/mL against two prokaryotic bacterial species, *S. aureus* and *E. coli*. Both composites showed higher inhibition activity. Their inhibition activity was confirmed by measuring diameter of inhibition zone. Again their inhibition activity increased as the concentration increased. In case of GO-PANI-Mupirocin composite the inhibition zone diameter was slightly higher than GO-PANI due to the addition of Mupirocin. This implied that both the bacteria were susceptible to the antibiotic Mupirocin. The minimum inhibition zone diameter was 13.5 mm and maximum was 27mm that means the synthesized samples showed greater antibacterial activity. Since all specimens inhibited the microbial growth which proved that they can be act as useful antibacterial agent for different microorganisms. These composites may be helpful for the future drug design.

References

[1] A.M. Allahverdiyev, E.S. Abamor, M. Bagirova, M. Rafailovich, "Antimicrobial effects of TiO_2 and Ag_2O Nano particles against drug-resistant bacteria and leishmania parasites", *Future Microbiology,* vol. 6, pp. 933-940, 2011.

[2] S. Stankovich, "Graphene-based composite materials", *Nature,* vol. 422, pp. 282-286, 2006.

[3] A. Lerf, H.Y. He, M. Forster, J. Klinowski, "Structure of graphene oxide revisited", *J. Phys. Chem. B,* vol. 102, pp. 4477-4482, 1998.

[4] G. Eda, G. Fanchini, M. Chhowalla, "Large-area ultrathin films of reduced graphene oxide as a transparent and flexible electronic material", *Nat. Nanotechnol,* vol. 3, pp. 270-274, 2008.

[5] Y.B. Zhang, S.F. Ali, E. Dervishi, Y. Xu, Z.R. Li, "Cytotoxicity effects of graphene and single-wall carbon nanotubes in neural phaeochromocytoma-derived PC12 cells", *ACS Nano,* vol. 4, pp. 3181-3186, 2010.

[6] J. Tang, Q. Chen, L. Xu, S. Zhang, L. Feng, "Graphene oxide-silver nanocompositeas a highly effective antibacterial agent with speciesspecific mechanisms", *ACS Appl. Mater. Interfaces,* vol. 5, pp. 3867-3874, 2013.

[7] C.M. Santos, J. Mangadlao, F. Ahmed, A. Leon, R.C. Advincula, "Graphene nanocomposite for biomedical applications: fabrication, antimicrobial and cytotoxic investigations", *Nanotechnology,* vol. 23, pp. 39-51, 2012.

[8] A.F.D. Faria, A.C.M.D. Moraes, O.L. Alves, "Toxicity of Nanomaterials to Microorganisms: Mechanisms, Methods, and New Perspectives", *Nanomedicine and Nanotoxicology,* vol. 1, pp. 363-405, 2014.

[9] S. Liu, T.H. Zeng, M. Hofmann, E. Burcombe, J. Wei, R. Jiang, J. Kong, Y. Chen, "Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress", *ACS Nano,* vol. 5, pp. 6971-6980, 2011.

[10] R.D. Manash, K.S. Rupak, S. Ratul, S.K. Vinayak, V.S. Manjusha, S. Pinaki, "Synthesis of silver nanoparticles in an aquaeous suspension of grapheme oxide sheets and its antimicrobial activity", *Colloids and surfaces B. Biointerfaces,* vol. 83, pp. 16-22, 2011.

[11] C. Harish, V. Sai SreeHarsha, C. Santhosh, R. Ramachandran, M. Saranya, T.M. Vanchinathan, K. Govardhan, A. Nirmala Grace, "Synthesis of Polyaniline/Graphene nanocomposites and its optical, electrical and electrochemical properties", *Adv. Sci. Eng. Med.,* vol. 4, pp. 1-9, 2012.

[12] C. Gelmetti, "Local antibiotics in dermatology", *Dermatol. Therapy,* vol. 21, pp. 187-195, 2008.

[13] B.D. Cookson, "The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice", *J. Antimicrob. Chemother.,* vol. 41, pp. 11-18, 1998.

[14] P.J. O'Hanlon, N.H. Rogers, J.W. Tyler, "The chemistry of pseudomonic acid. Part 6. Structure and preparation of pseudomonic acid D", *J. Chem. Soc. Perk.,* vol. 4, pp. 2655-2657, 1983.

[15] L. Shahriary, A.A. Athawale, "Graphene Oxide Synthesized by using Modified Hummers Approach", *Int. J. Ren. En. Env. Eng.,* vol. 2, pp. 58-63, 2014.

[16] E.C. Gomes, M.A.S. Oliveira, "Chemical Polymerization of Aniline in Hydrochloric Acid (HCl) and Formic Acid (HCOOH) Media. Differences between the Two Synthesized Polyanilines", *American Journal of Polymer Science,* vol. 2, No. 2, pp. 5-13, 2012, DOI: 10.5923/j.ajps.20120202.02

[17] K.P. Jotiram, R.G.S.V. Prasad, V.S. Jakka1, R.S.L.Aparna, A.R. Phani, "Antibacterial Activity of Nanostructured Polyaniline Combined With Mupirocin", *Nano. Biomed. Eng.,* vol. 4, pp. 144-149, 2012.

[18] J. Zhu, M. Chen, H. Qu, X. Zhang, H. Wei, Z. Luo, H. A. Colorado, S. Wei, Z. Guo, "Interfacial polymerized polyaniline/graphite oxide nanocomposites toward electrochemical energy storage", *Polymer,* vol. 53, pp. 5953-5964, 2012.