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Biological evaluation of nano composites derived from poly methyl metha acrylate and NiO / Al_2O_3 dimetal oxides by ashweghanda extract

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Abstract

This study employed a green route to synthesize metal oxide nanocomposites, specifically NiO/Al_2O_3, which were subsequently used to dope poly(methyl methacrylate) (PMMA) as a matrix, resulting in the formation of novel nanocomposites, namely PMMA/NiO/Al_2O_3. The nanocomposites were characterized using FE-SEM, XRD, DSC, TGA, antioxidant activity, and antibacterial activity tests to confirm their design, verify the nanostructured composition, and distinguishable features in poly methyl methacrylate distribution. The results of AFM for the synthesized nanostructured showed that the NiO and Al_2O_3, describe how the nanoparticles' size was in the nano scale. The biological activities of nanocomposites synthesized were studied, including antioxidant and the use of these nanocomposites as inhibitors for bactria also as anticancer. The findings for all of these nanocomposites revealed they have the properties of a significant inhibitory effect.

Keywords: Poly methyl metha acrylate , nikel oxide ,alumminum oxide, Antioxidant activity , antibacterial activity and anticancer activity.

تقييم بيولوجي لمركبات النانو التي تنحدر من أكريلات الميثيل المتعددة وأكاسيد ثنائية المعدن (NiO / Al_2O_3) التي تنتجها خلاصة اشواغاندا

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الخلاصة

استخدمت هذه الدراسة طريقة خضراء لتصنيع مركبات نانوية من أكسيد المعادن، وتحديدًا NiO/Al_2O_3، والتي استخدمت لاحقًا لتخدير بولي (ميثيل ميثاكريلات) (PMMA) كمصفوفة، مما أدى إلى تكوين مركبات نانوية جديدة، وهي PMMA/NiO/Al_2O_3. تم توصيف المركبات النانوية باستخدام اختبارات FE-SEM وXRD وDSC وTGA والنشاط المضاد للأكسدة والنشاط المضاد للبكتيريا لتأكيد تصميمها والتحقق من التركيب النانوي والسمات المميزة في توزيع بولي ميثيل ميثاكريلات. أظهرت نتائج AFM للمركبات النانوية المصنعة أن NiO وAl_2O_3 يصفان حجم الجسيمات النانوية على نطاق النانو. تمت دراسة الأنشطة البيولوجية للمركبات النانوية المصنعة، بما في ذلك مضادات الأكسدة واستخدام هذه المركبات النانوية

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كمثبطات للبكتيريا وأيضًا كمضاد للسرطان. كشفت النتائج لجميع هذه المركبات النانوية عن امتلاكها لخصائص ذات تأثير مثبط كبير.

1. Introduction

The process of hybridization is often essential when combining two or more distinct materials to achieve a synergy of desirable properties. This approach is particularly valuable in the development of polymers with specialized functions and tailored performance characteristics [1]. Polymer composites can be produced using a large variety of organic or inorganic additive materials, depending on performance requirements for desired application. Although composite materials are also frequently used to describe the hybridization of materials, polymer composites can also contain hybrid additives and fillers [2]. The polymerization pathways and the interactions between the substituents are crucial in defining the characteristics and functions of the resulting polymer composites, in addition to the appropriate selection of each individual material.

In general, there are various approaches to synthesizing functionalized polymeric hybrid/composite synthesis; these methods depend on the final properties and applications of the material, as well as the inclusion of additional monomers, materials, and solvents. The final properties of the synthesized materials are influenced by the polymerization parameters, which also impact molecular weight, solubility in water and solvents, particle shape and morphology, surface modification, and polymer grafting. To create polymeric materials with certain qualities, more than one monomer may be added during the polymerization process, as well as the addition of nanoparticles. This structure is frequently called a nanohybrid material [3] or, in this context, nanohybrid polymer composites (NHPs). A variety of properties, such as improved polymer elastic performance, outstanding thermal stability, improved wettability, remarkable photostability, electrical conductivity, and ultimately improved system properties and functionalities, can be achieved by producing NHPs by surface-modifying nanoparticles onto polymers [4-7]. Polymeric batteries, medication delivery systems, building, architecture, and wastewater treatment are common uses that help NHPs.

Poly(methyl methacrylate) (PMMA) is a widely utilized polymer in the fabrication of nanohybrid polymers (NHPs) or polymer composites. PMMA is frequently combined with nanoparticles. Usually, the PMMA matrix's performance and usefulness are improved by adding nanoparticles to it. Methyl methacrylate monomer and a free radical initiator are used to create PMMA, which is a simple, hassle-free, and inexpensive process. PMMA depolymerizes between 300 and 400 °C, producing MMA's volatile monomer [8]. With outstanding mechanical, optical, and thermal stability, PMMA is available as a liquid and solid resin. In particular, these characteristics include strong UV absorption, fungal resistance, flexural strength, thermal stability, and transmittance value, can be enhanced when nanoparticles are incorporated into the system [9-11]. PMMA is employed in many different applications spanning optical materials, automotive, electronics, displays, and other industries because it is stiff, lightweight, and versatile in terms of color [12].

Recent studies have shown that nanoparticles and materials containing nanoparticles or nanostructures consisting of particles or structures between 1 to 100 nm, exhibit high strength, high surface area, and low weight. One of the most notable characteristics of nanoparticles is that they have a broad absorption spectrum and, depending on their characteristics and shape, a large surface area. Nanoparticles range in size from 1 and 100 nm, and nanomaterials are the link between atoms and microstructures, indicating that they are close to atomic dimensions. Also, they are reasonably priced and retain all of their light activity even after repeated use [13].

Metal oxides at the nanoscale possess two remarkable properties - a high removal capacity for heavy metals as well as selectivity towards certain types of heavy metals. As a result, metal oxide nanomaterials show great promise as heavy metal adsorbents. Examples of metal oxide-based nanomaterials include manganese oxides, nano-sized iron oxides, titanium oxides, cerium oxides, ZnOs, magnesium oxides, aluminum oxides, and zirconium oxides [14].

Researchers from all around the world are very interested in NiO NPs because they are essential semiconductors with a large band gap and magnetic characteristics. Of all the transition metal oxides, nickel oxide has exceptional electrochemical properties that make it a viable option for use in supercapacitors [15].

The aim of the study involved development of a straightforward green method for synthesizing manganese and nickel in the [PMMA / NiO / Al_2O_3] nanocomposite. Characterization encompassed evaluating its SEM, antioxidant properties, and antibacterial activities. Antibacterial efficacy was assessed using the disc diffusion method against reference strains of both gram-positive [*Staphylococcus aureus (+)*] and gram-negative [*Escherichia coli (-)*] bacteria, demonstrating direct inhibitory action. Furthermore, the antioxidant ability of nanocomposites and SEM characteristics were thoroughly investigated.

2. Experiential part

2.1 Materials

Poly methyl metha acrylate, Ashweghanda extract solution**,** alumminum oxide, nikel oxide were supplied by BDH.

2.2 Preparation of ashweghanda aqueous extract [16]

Generally, 4 g of ashweghanda extract were mixed with 150 mL of distilled water. Prior to filtration, the liquid was heated in order to be used for synthesis while stirring continuously for a duration of 15 minutes.

2.3 Synthesis of Aluminum (NPs) in green method [17]

4.0 gm of Ashweghanda extract was added to 100 mL of distilled water, and the mixture was then heated at 80 °C for 15 min before being filtered. In a 250 mL vessel, deionized water was added and heated to 80 °C with continuous stirring for 15 minutes. Subsequently, 6 grams of aluminum chloride (AlCl3) were added to the solution. Notably, the addition of ashweghanda extract via dropwise addition, accompanied by stirring, resulted in a distinct color change of the AlCl3 solution, transitioning from white to brown. The mixture was heated at 100 °C, until the solvent was evaporated. After obtaining the precipitate by drying the solution at 60 °C, allow it cool to room temperature.

2.4 Synthesis of Nikel (NPs) in green method [17]

4.0 gm of Ashweghanda extract was added to 100 mL of distilled water, and the mixture was heated at 80 °C for 15 min before filtering. Six grams of [$Ni(NO_3)_2$] were added to 250 mL of deionized water. The mixture was heated at 80 °C and stirred for 15 min. When ashweghanda extract was added dropwise to the mixture, the color of the [$Ni(NO_3)_2$] solution turned from green to dark green with stirring. Following, the mixture was heated at 100 °C till the solvent was completely evaporated. Finally, the obtained precipitate was dried at 60 °C and allowed to cool at room temperature.

2.5 Preparation of [PMMA / NiO / Al_2O_3] nanocomposites [18]

A 1.0 gm of polystyrene was mixed with 25 mL of acetone, and refluxed for 1 hour at 50 °C (solution A). A 0.1 gm of Ni nanoparticles and 0.1 gm of Al nanoparticles were mixed in 10 mL of acetone. The mixture was then placed in an ultrasonic cleaner for five minutes (solution B). After mixing the both solutions (A and B), the mixture was refluxed for 5.0 hrs. The mixture was collected and allowed to solidify at room temperature.

3. Results and Discussion

3.1 Field emission scanning electron microscopy (FE-SEM) of (PMMA \ NiO \ Al_2O_3) nanocomposites

Field Emission Scanning Electron Microscopy (FE-SEM) is an analytical technique that is used in materials science to study the surfaces morphology of martials [19]. The FE-SEM measurements revealed that the nanoparticles have an irregular shape with bone-like characteristics that ranged in size from 44.66 to 180.0 nm and a sheet with a thickness of around 4.3 nm (the presence of microscopic and nanosized particles opens up opportunities for innovation across various fields, driven by their unique properties and potential applications in enhancing materials, technology, medicine, and environmental sustainability); these dimensions fall under the definition of the nano scale, as shown in Figure 1.

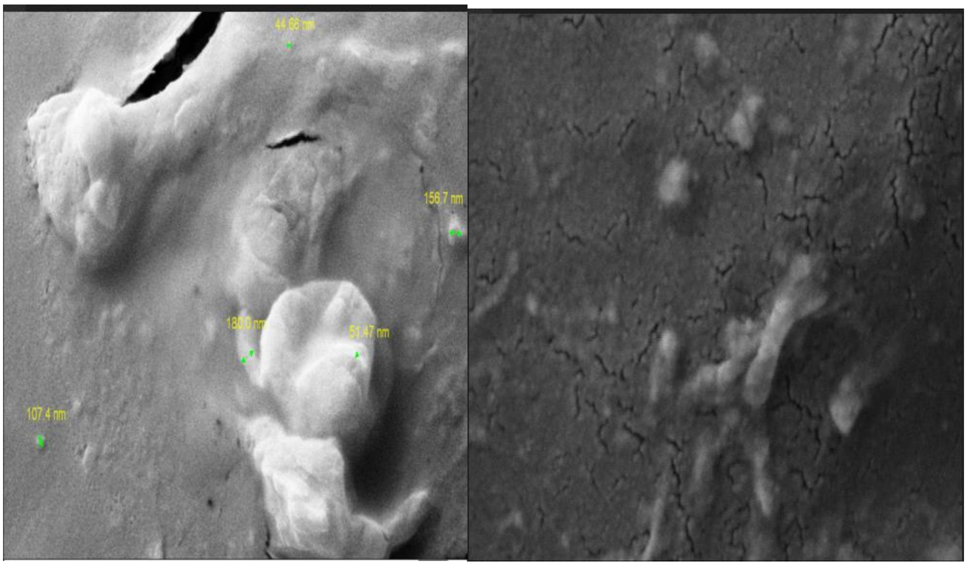

Figure 1: FE-SEM of (PMMA \ NiO \ Al_2O_3) nanocomposites

3.2 X-ray diffraction (XRD) of (PMMA \ NiO \ Al_2O_3) nanocomposites

X-ray diffraction (XRD) stands out as a crucial and widely adopted technique for material characterization [20]. XRD pattern of (PMMA \ NiO \ Al_2O_3) nanostructure demonstrate that the peaks exhibited diffraction peaks at the 2θ values (14°, 27°, 30°,31°, 32°,45°, 57°,67, and 76°). However, the mean crystal size of (PMMA \ NiO \ Al_2O_3) nanoparticles was 29.42 nm.

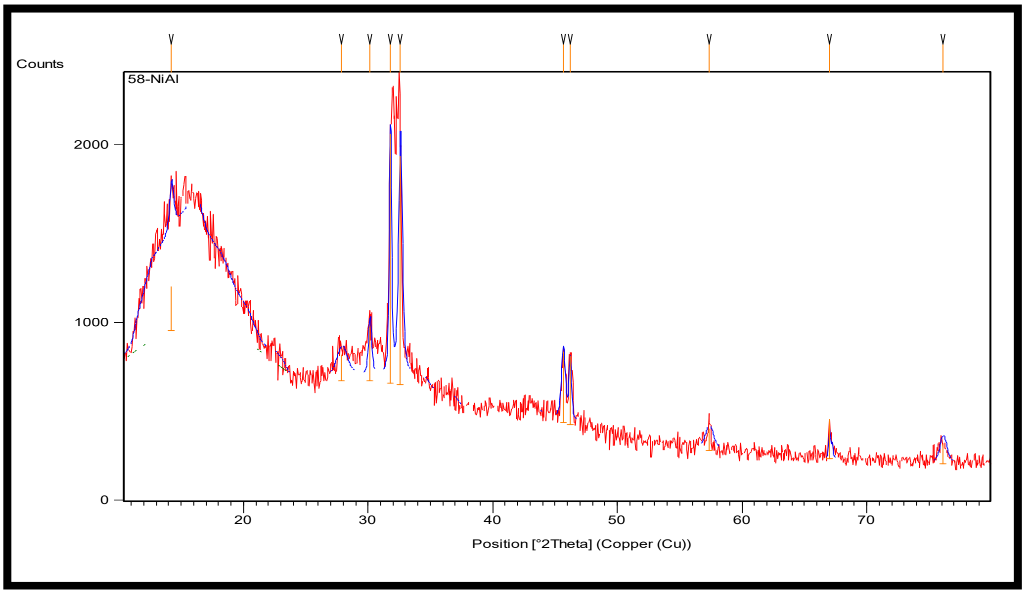

Figure 2: XRD pattern of (PMMA \ NiO \ Al_2O_3) nanocomposites.

3.3 Thermogravimetric (Tg) analysis of polymethylmehtaacrylate (PMMA \ NiO \ Al_2O_3) nanocomposites.

Tg analysis of synthesized nanocomposites showed four stages of weight loss [21] :

1- The first stage occurred between 35 and 120 °C, with a percentage of weight loss of 8.438%. This was ascribed to the physical adsorption of water (taken from the ambient) into the nanocomposites because the nanomaterials have a very large surface area [21].

2- The second stage occurs between 120 and 440 °C, while the third stage occurs between 440 and 800 °C. The overall weight (percentage) loss during these stages is equivalent to 55.39%. Acrylate unit loss begins at these temperatures, and the polymer's carbon backbone begins to break down.

3- The fourth stage, which occurred at a temperature of more than 800 °C and a weight (percentage) of 12.18%. See table1.

Table 1: Display all stage breakdowns of the (PMMA \ NiO \ Al_2O_3) nanocomposite

Ser	*Stage (temp) °C*	*Loss wt %*	*status*
1	35-120	8.438	Water loss
2	120-440	22.54	beginning of the acrylate unit's loss
3	440-800	32.85	Carbon skeleton breakdown
4	More than 800	12.18	remnant of metal and metal oxide

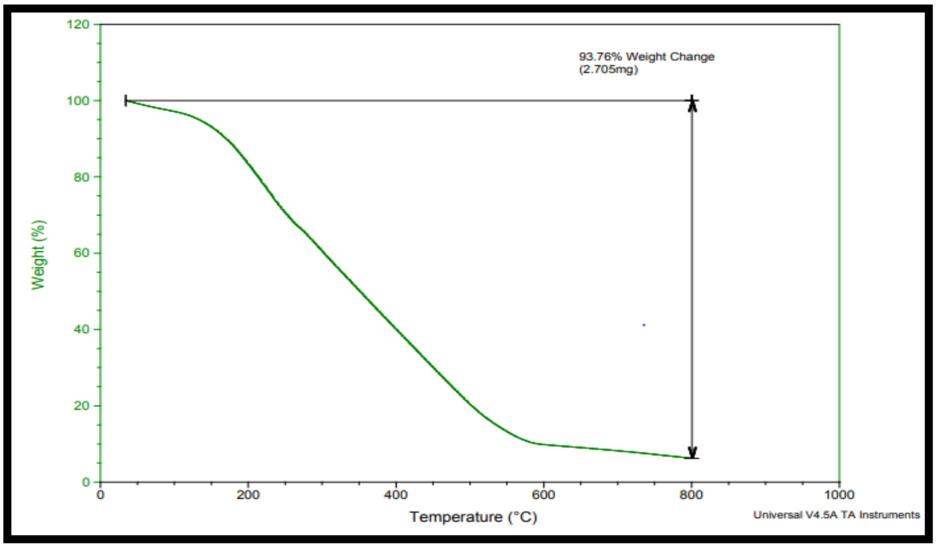

Figure 3: TGA analysis of (PMMA \ NiO \ Al_2O_3) nanocomposites

3.4 Differential scanning calorimetry (DSC) analysis of (PMMA \ NiO \ Al_2O_3) nanocomposites.

Differential Scanning Calorimetry (DSC), a thermal analysis technique, enables the measurement of the heat capacity of a sample by detecting the amount of energy absorbed or emitted as heat during thermal transitions. DSC equipment can be used to calculate the thermal capacity, enthalpy, entropy, and phase diagram [22]. Two endothermic phases were identified in the prepared (PMMA\NiO\$Al_2O_3$) nanocomposites by DSC. First, at 346.68°C, which is equivalent to AH to 345.1 J/g for (PMMA\NiO\$Al_2O_3$), it is considered that PMMA in nanocomposites has reached its the crystalline melting point when compared to the standard reference of 100°C. Secondly, at 439.10°C, 388.58°C, 438.11°C, and 399.22°C, respectively. Furthermore, it was noted that PMMA in nanocomposites has reached its melting point when compared to the standard reference of 165 °C, which shows the effectiveness of the response, as seen in fFigure 4.

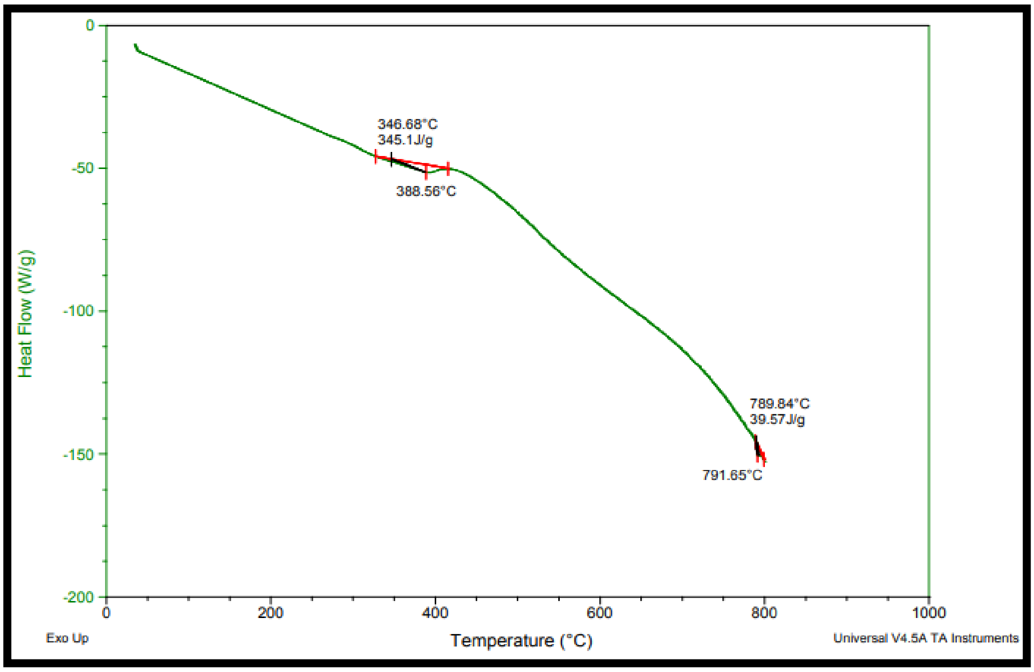

Figure 4: DSC/TGA of (PMMA \ NiO \ Al_2O_3) nanocomposites.

4. Application

4.1 Antioxidant of (PMMA \ NiO \ Al_2O_3) nanocomposites

A. Solutions [23]

4.3 mg of DPPH (1,1-Diphenyl-2-picryl-hydrazyl) was dissolved in 3.3 mL of DMSO-methanol (1:9 v/v), and the test tubes were covered with aluminum foil to prevent light from reaching the solution. Following, four concentrations 0.0625, 0.125, 0.250 and 0.500 mg/mL were prepared by dissolving the required weight of methanolic extract in 1-2 drops of DMSO, and then the volume was reached to the mark with distilled water. Similar concentrations of ascorbic acid (vitamin C) were prepared from the plant extract.

B. Method

The antioxidant activity of the plant methanolic extract and the standard (vitamin C) was assessed by evaluating the ability of the prepared DPPH free radical to scavenge radicals, following the methodology outlined by Sanja et al.[23]. A 3.9 mL of exacted solution in DPPH was placed in a test tube together with a portion of 0.1 mL of the extract or standard (0.625, 0.125, 0.250, and 0.500 mg/mL). The absorbance of each solution was measured at 517 nm using a spectrophotometer following a 30 min incubation period at 37°C. Three duplicate measurements were conducted for each solution. Eq. (1) was used to determine the capacity to scavenge DPPH radical.

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}\right) \times 100 \qquad (1)$$

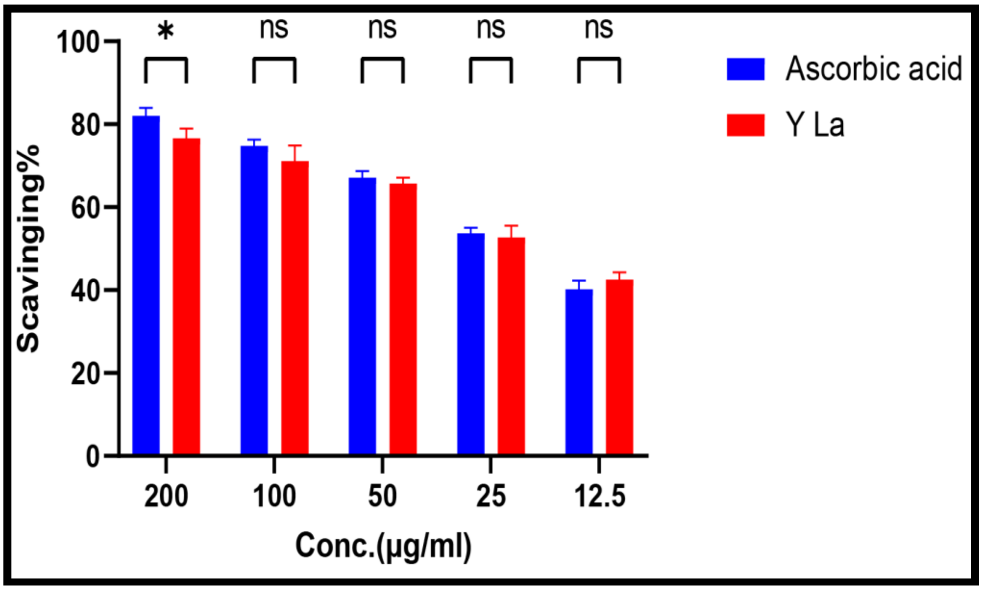

Figure 5: Antioxidant of (PMMA \ NiO \ Al_2O_3) nanocomposites

4.2 Antibacterial activity of (PMMA \ NiO \ Al_2O_3) nanocomposites

As observed in Figure 6, the biological results on the bacteria *E. coli (G-), S. aureus (G+), pseudomonas (G-), and streptococcus (G+)* demonstrated that nanocomposites have distinct effects on preventing the growth of the bacteria under study. This is because these nanocomposites can produce free radicals, which can cause oxidative stress and damage to proteins, DNA, and cell membranes. binding to cytosolic DNA, enzymes, and proteins. Reduced inhibition of the respiratory chain, ATP synthesis, and metabolic pathways results from this interaction [24].

The nanocomposites exert their antibacterial effect by disrupting the bacterial cell membrane's function through a two-pronged mechanism: electrostatic binding, where the positively charged nanocomposites interact with the negatively charged membrane, and the release of positively charged metal ions, which further compromise the membrane's integrity. These charges cause electrostatic binding to interfere on the surface, increasing oxidative stress and damaging the cell membrane [25].

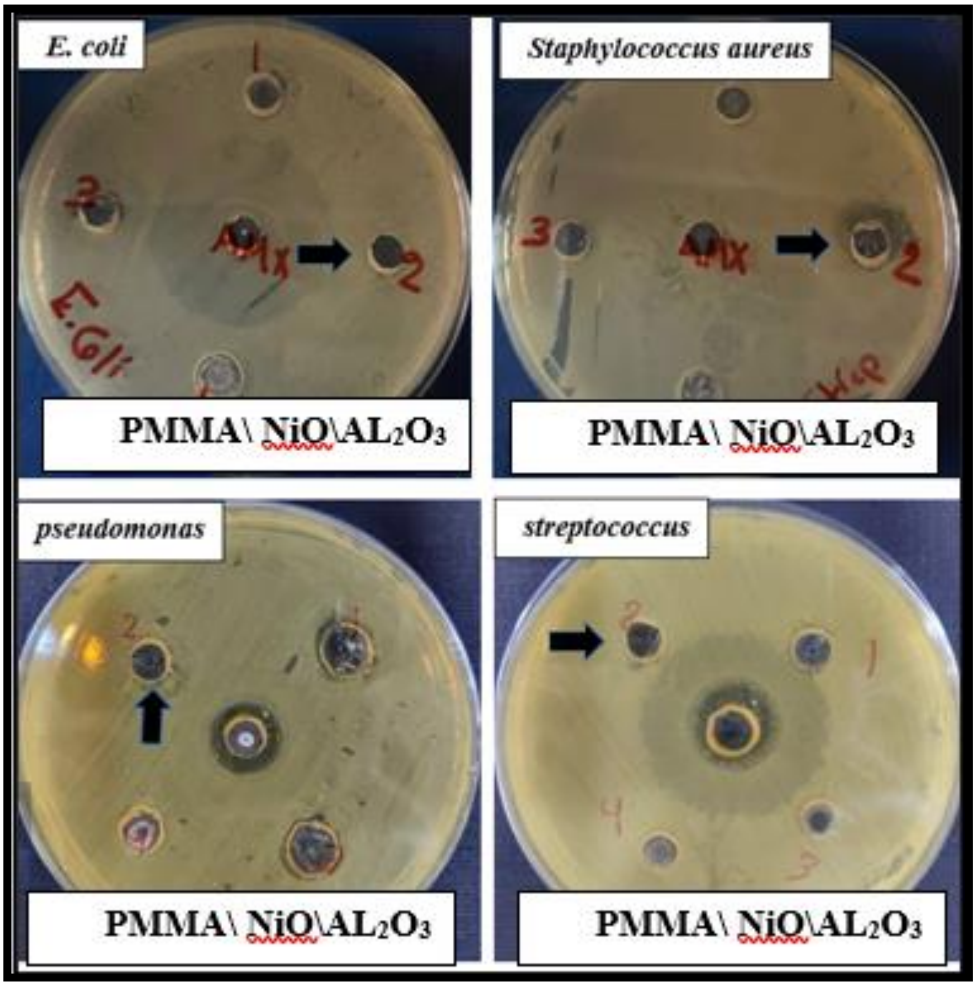

Figure 6: Antibacterial activity of (PMMA \ NiO \ Al_2O_3) nanocomposites on the bacteria *E. coli, Staphylococcus aureus, pseudomonas*, and *streptococcus* demonstrated that nanocomposites

4.3 Anticancer activity of (PMMA \ NiO \ Al_2O_3) nanocomposites.

The toxicity of (PMMA \ NiO \ Al_2O_3) nanocomposites (made in an environmentally friendly manner) on cells was assessed using the MTT test. Utilizing six distinct concentrations and three duplicates of each concentration, the MTT test was used to determine the rate of inhibition and cell viability on the breast cancer cell line MDA-MB-231. Assembled test plate (7) for the MTT technique it is also natural to compare them. The six concentrations of [PMMA \ NiO \ Al_2O_3] on MCF-7 demonstrated the harmful effect on the cells. Table 2 indicates that an increase in [PMMA \ NiO \ Al_2O_3] concentration resulted in a decrease in the cell's viability and capacity.

Table 2: Cytotoxicity effect of [PMMA \ NiO \ Al_2O_3] after 24 hours incubation at 37ºC

Concentration (ppm)	*Mean*	*SD*
1000	51.77778	2.0367
500	45.11111	2.694301
250	38.22222	2.0367
125	23.33333	2.666667
62.5	18.66667	2.403701
31.25	13.11111	2.0367

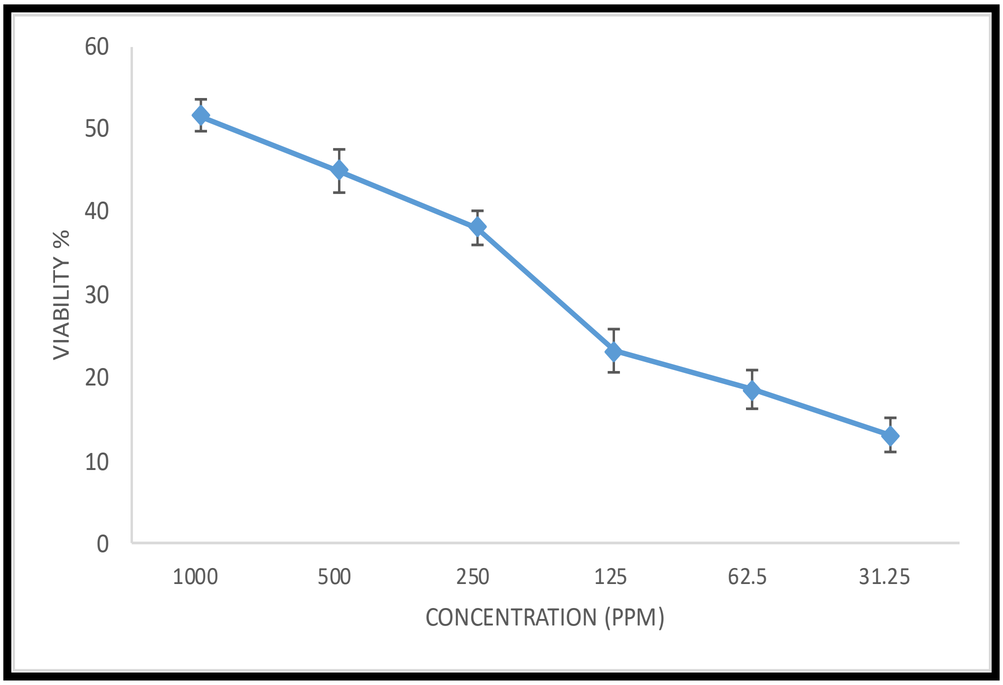

Figure 7: Cytotoxicity effect of [PMMA \ NiO \ Al_2O_3] after 24 hours incubation at 37ºC

Conclusion

Utilizing ashweghanda extract solution, Nikel, and allminum oxide nanoparticles, the Poly methyl metha acrylate [PMMA \ NiO \ Al_2O_3] nanocomposites was synthesized. It was then combined with polymethyl metha acrylate that had been dissolved in acetone as a solvent. The FESEM technique showed that metal oxide nanoparticles developed as a coating layer over and between polymethyl methacrylate, XRD analysis was conducted, and PMMA nanoparticles have a cubic shape. The antibacterial and antioxidant activities were assessed at the site where microbial growth was inhibited, while evaluating their effectiveness against cancerous cells. Significant antioxidant capability was observed through effective scavenging of free radicals and anti-cancer activity. The antioxidant and anti-cancer properties associated with PMMA/NiO/Al_2O_3 nanocomposites primarily stem from the inherent properties of the nanoparticles (NiO and possibly Al_2O_3) rather than from PMMA itself. The effective functional groups in PMMA, such as ester groups, contribute to the overall structure and properties of the nanocomposite material but do not directly confer antioxidant or anti-cancer effects.

References

[1] W. Zhang and A. H. Müller, "Architecture, self-assembly and properties of well-defined hybrid polymers based on polyhedral oligomeric silsequioxane (POSS)," *Progress in Polymer Science,* vol. 38, no. 8, pp. 1121-1162, 2013.

[2] Y. Swolfs, L. Gorbatikh, and I. Verpoest, "Fibre hybridisation in polymer composites: A review," *Composites Part A: Applied Science and Manufacturing,* vol. 67, pp. 181-200, 2014.

[3] S. Kaur, M. Gallei, and E. Ionescu, "Polymer–ceramic nanohybrid materials," *Organic-Inorganic Hybrid Nanomaterials,* pp. 143-185, 2015.

[4] L. M. Corredor, M. M. Husein, and B. B. Maini, "A review of polymer nanohybrids for oil recovery," *Advances in colloid and interface science,* vol. 272, p. 102018, 2019.

[5] J. Sun, Q. Ma, D. Xue, W. Shan, R. Liu, B. Dong, J. Zhang, Z. Wang, and B. Shao, "Polymer/inorganic nanohybrids: An attractive materials for analysis and sensing," *TrAC Trends in Analytical Chemistry,* vol. 140, p. 116273, 2021.

[6] J. Zia and U. Riaz, "Photocatalytic degradation of water pollutants using conducting polymer-based nanohybrids: A review on recent trends and future prospects," *Journal of Molecular Liquids,* vol. 340, p. 117162, 2021.

[7] S. Prakash, M. Malhotra, W. Shao, C. Tomaro-Duchesneau, and S. Abbasi, "Polymeric nanohybrids and functionalized carbon nanotubes as drug delivery carriers for cancer therapy," *Advanced drug delivery reviews,* vol. 63, no. 14-15, pp. 1340-1351, 2011.

[8] O. S. Manoukian, N. Sardashti, T. Stedman, "*Biomaterials for tissue engineering and regenerative medicine,*" 2019.

[9] A. Alsaad, Q. M. Al-Bataineh, A. Ahmad, I. Jum'h, N. Alaqtash, and A. Bani-Salameh, "Optical properties of transparent PMMA-PS/ZnO NPs polymeric nanocomposite films: UV-Shielding applications," *Materials Research Express,* vol. 6, no. 12, p. 126446, 2020.

[10] R. Giti, M. Firouzmandi, N. Zare Khafri, and E. Ansarifard, "Influence of different concentrations of titanium dioxide and copper oxide nanoparticles on water sorption and solubility of heat-cured PMMA denture base resin," *Clinical and Experimental Dental Research,* vol. 8, no. 1, pp. 287-293, 2022.

[11] U. Mangal, J.-Y. Kim, J.-Y. Seo, J.-S. Kwon, and S.-H. Choi, "Novel poly (methyl methacrylate) containing nanodiamond to improve the mechanical properties and fungal resistance," *Materials,* vol. 12, no. 20, p. 3438, 2019.

[12] U. Ali, K. J. B. A. Karim, and N. A. Buang, "A review of the properties and applications of poly (methyl methacrylate)(PMMA)," *Polymer Reviews,* vol. 55, no. 4, pp. 678-705, 2015.

[13] S. S. Salem, E. N. Hammad, A. A. Mohamed, and W. El-Dougdoug, "A comprehensive review of nanomaterials: Types, synthesis, characterization, and applications," *Biointerface Research in Applied Chemistry,* vol. 13, no. 1, p. 41, 2022.

[14] Z. Zaid Almarbd and N. Mutter Abbass, "Synthesis and characterization of TiO2, Ag2O, and graphene oxide nanoparticles with polystyrene as a nonocomposites and some of their applications," *Eurasian Chem. Commun,* vol. 4, pp. 1033-1043, 2022.

[15] O. Nimisha, A. R. Mary, and R. Ramanarayanan, "Template assisted green synthesis of nickel oxide nanoparticles for photocatalytic applications," in *IOP Conference Series: Materials Science and Engineering*, 2022, vol. 1258, no. 1: IOP Publishing, p. 012010.

[16] A. El-Ansary, A. Warsy, M. Daghestani, N. M. Merghani, A. Al-Dbass, W. Bukhari, B. Al-Ojayan, "Characterization, antibacterial, and neurotoxic effect of green synthesized nanosilver using Ziziphus spina Christi aqueous leaf extract collected from Riyadh, Saudi Arabia," *Materials Research Express,* vol. 5, no. 2, p. 025033, 2018.

[17] R. Khani, B. Roostaei, G. Bagherzade, and M. Moudi, "Green synthesis of copper nanoparticles by fruit extract of Ziziphus spina-christi (L.) Willd.: Application for adsorption of triphenylmethane dye and antibacterial assay," *Journal of Molecular Liquids,* vol. 255, pp. 541-549, 2018.

[18] W. Ahmad, Q. Ahmad, M. Yaseen, I. Ahmad, F. Hussain, B. Mohamed Jan, R. Ikram, "Development of waste polystyrene-based copper oxide/reduced graphene oxide composites and their mechanical, electrical and thermal properties," *Nanomaterials,* vol. 11, no. 9, p. 2372, 2021.

[19] N. Brodusch, H. Demers, and R. Gauvin, *Field emission scanning electron microscopy: New perspectives for materials characterization*. Springer, 2018.

[20] A. Ali, Y. W. Chiang, and R. M. Santos, "X-ray diffraction techniques for mineral characterization: A review for engineers of the fundamentals, applications, and research directions," *Minerals,* vol. 12, no. 2, p. 205, 2022.

[21] T. Remiš, P. Bělský, T. Kovářík, J. Kadlec, M. Ghafouri Azar, R. Medlín, V. Vavruňková, "Study on structure, thermal behavior, and viscoelastic properties of nanodiamond-reinforced poly (vinyl alcohol) nanocomposites," *Polymers,* vol. 13, no. 9, p. 1426, 2021.

[22] B. H. Soudmand, K. Shelesh-Nezhad, and Y. Salimi, "A combined differential scanning calorimetry-dynamic mechanical thermal analysis approach for the estimation of constrained phases in thermoplastic polymer nanocomposites," *Journal of Applied Polymer Science,* vol. 137, no. 41, p. 49260, 2020.

[23] S. Sanja, N. Sheth, N. Patel, D. Patel, and B. Patel, "Characterization and evaluation of antioxidant activity of Portulaca oleracea," *International Journal of Pharmacy and Pharmaceutical Sciences,* vol. 1, no. 1, pp. 74-84, 2009.

[24] C. Völker, M. Oetken, and J. Oehlmann, "The biological effects and possible modes of action of nanosilver," *Reviews of Environmental Contamination and Toxicology,* vol. 223, pp. 81-106, 2012.

[25] L. Wang, C. Hu, and L. Shao, "The antimicrobial activity of nanoparticles: present situation and prospects for the future," *International journal of nanomedicine,* pp. 1227-1249, 2017.