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Enhancing of Antibacterial Activity of Polyurethane (TPU) Nanofiber via Electrospinning Technique Using Green Extracted Zinc Oxide (ZnO NPs) and Graphene Oxide (GO NPs)

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Abstract

Some harmful compounds could be dangerous if used in the medical field, Physical, chemical, and green synthesis processes produce metal oxide nanoparticles, this research aims to study extracting Zinc Oxide nanoparticles in a green way using orange fruit peel, to reduce using toxic chemicals in nanoparticle invention and enhance the antibacterial activity, biological and medical applications of Zinc Oxide/Graphene Oxide nanoparticles. **Methods:** The study utilized the electrospinning method to fabricate the Polyurethane nanofibers, and a green synthesis approach was employed to produce zinc oxide. Structural properties of Zinc Oxide/Graphene Oxide composite nanomaterials were characterized with X-ray diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Transmission Electron microscope (TEM). **Results:** the results of the study indicate that ZnO can be synthesized in a green way from orange peel and ZnO/GO-embedded polyurethane nanofibers exhibited strong antibacterial activity toward Escherichia coli (*E. coli*). As evidenced by the observed SEM and TEM analysis, FTIR spectroscopy, and XRD characterization. **Conclusion:** The research findings suggest that using polyurethane nanofibers via electrospinning could be an applicable curriculum for addressing the challenges associated with antibacterial activity and eco-friendly compounds in the medical field.

Keywords: Green synthesis, Zinc Oxide (ZnO), Graphene Oxide (GO), Polyurethane (TPU), Electrospinning.

Introduction

Organic antibacterial agents are sensible to conditions such as temperature and pressure, in current years, inorganic antibacterial agents are the trend of research concerned for microbes. The effect of metal-oxide materials (inorganic) is great at multiple concentrations proven against bacteria. One of their features is that they maintain the stability of the composition at high temperature and pressure and are suitable for use for long periods**[1]**. Metal oxide nanoparticles (ZnO) are produced by physical, chemical, and green synthesis processes. According to the literature, some harmful compounds we employ in physical and chemical procedures may be present in the generated metal oxide nanoparticles, which could be dangerous if used in the medical area. ZnO NPs can be produced through many techniques. As sol-gel, thermal evaporation, microfluidics, and mechanochemical methods. Microwave technique syntheses also have many advantages like shorter reaction time, better size, and morphology control regarding traditional chemical methods [16]. Moreover, these methods use harmful chemicals like solvents and reducing agents, which harm the environment. So, to preserve the environment, zinc oxide in nanoform is synthesized by green synthesis processes **[**5**]**. The most common method for ZnO in nanocomposite preparation is the wet chemical process, because it is often cheaper, simpler, and produces higher amounts of nanoparticles. It involves the reaction of a zinc precursor, usually zinc nitrate, zinc chloride, zinc acetate, or zinc sulfate, with a base solution such as Na OH or KOH. Recently, a growing attention in nanomaterials, as the use of green composites to synthesize ZnO, such as plant extracts, to replace NaOH and KOH as reducing and capping agents. Extracts can also functionalize nanoparticles with phytochemicals that have antioxidant, antimicrobial, or anti-inflammatory properties, further alleviating potential toxicity issues [16,17]. Besides, these kinds of preparations need a lot of energy and contain hazardous substances that pose a risk to biological systems. Metal oxide nanoparticles can be synthesized using environmentally friendly reducing and capping agents

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that prevent agglomeration, such as plant extracts. These methods are nowadays preferred over chemical and physical methods because they are non-toxic and less costly[11]. Zinc oxide can be applied for many applications, such as biosensors, cosmetics, drug carriers, and antibacterial agents[3]. Using microorganisms and plants with biomedical applications to produce nanoparticles (Green synthesis). There are many advantages like eco-friendliness, safety, cost reduction, and biocompatibility. Strong antibacterial properties have been proved by using ZnO NPs extracted with green synthesis processes[1]. Currently, ZnO nanoparticles are extracted from plants. Compared the bacterial properties of ZnO NPs synthesized from stem extract with those of chemically synthesized ZnO NPs. The result, the green synthesized Zinc Oxide NPs showed strong bacterial activity[11]. The most cultivated fruit in the world nowadays is the orange fruit. Orange fruit peel has many varieties of natural antioxidants. Therefore, we use the extract of orange peel as a stabilizer to prepare ZnO NPs. However, more studies are still running out on ZnO NPs green synthesis extracted from orange fruit peel. Now, a green process using orange fruit peel extract was applied to synthesize ZnO NPs and test the antibacterial activities[6]. Herein, graphene has received great attention for its remarkable properties: high thermal conductivity, superior mechanical strength, large specific surface area, and excellent mobility of charge carriers. Graphene integration with polymers, materials such as metal, and metal oxide could add special functions to them. on the graphene sheets, the absence of functional groups limits their interaction with polymers, metal, and metal oxides. Furthermore, there are still challenges because of graphene's hydrophobic nature and incompatibility with organic polymers and the homogeneous dispersion. To figure out this problem, progressed graphene oxide (GO) was an alternative to pristine graphene. GO is synthesized by introducing on the surface many hydrophilic functional groups. Graphene Oxide shows good dispersibility in polymer solutions[2], compared to other carbon materials like graphene and carbon nanotubes, Graphene provides suitable sites for polymeric molecules to interact with it by the hydrogen bonds. Accordingly, we can consider Graphene Oxide one of the most important derivatives of graphene. Graphene oxide has approved its bacterial activity as in previous studies[8]. As a facility in the medical field applications Polyurethane nanofiber preparation via Electrospinning technique embedded with ZnO/GO NPs and more searches on applications are still running in the future[14]. Also, the activity of ZnO/GO -TPU nanofiber antibacterial was tested against E. coli.

Materials and Methods

Materials

Zinc nitrate salt (Sigma-Aldrich, crystallized 99.0%, USA)**,** (Orange peel) for its high content of organic compounds, Graphene oxide (Sigma-Aldrich,2 mg/mL, USA), Poly Urethane (BASF Elastollan, TPU LP9175, made in German), Di methyl formamide (DMF)(Sigma-Aldrich,99.0%, USA) , Distilled water as the synthesis medium.

Methods

Preparation of Orange peel extracts

The extracts were obtained by thoroughly cleaning and drying orange fruits before thinly peeling them. Afterward, the peel underwent a 12-hour drying process in a food drier until it became completely dry then, it was carefully ground into a powder with a smooth texture. 1 g of the powder was added to individual glass containers, each filled with 50 mL of distilled water. The mixture was stirred for 3 hours. The mixtures were subjected for 60 minutes to a water bath at 60 °C after being macerated. The combinations were filtered, and the resulting extracts were stored in an argon environment for future use [1,18].

Preparation of Zinc Oxide nanoparticles

The liquid form of the orange peel extract was separated using a centrifuge at 6000 rpm. The outcome of this process is a top-notch extract that can be utilized for future experiments. In the process of synthesizing ZnO NPs, 15 ml of extract was gradually added to a solution containing 35 ml of Zinc nitrate salt (7 mM). The colloidal reaction was heated at 60 ◦C for 4 hours, with continuous stirring at 500 rpm. The ZnONPs underwent a noticeable change in hue, transitioning from colorless to brown. Following a 4-hour reaction phase, the resulting creamy-colored ZnONPs were carefully collected, cleaned, and subjected to centrifugation at 10,000 rpm. This process was repeated three times to ensure thorough purification. Figure (1) shows the Preparation of green synthesis of orange peel extracts and Figure (2) displays the possible reaction mechanism of the Zinc oxide (ZnO) manufacturing process using orange peel extract. The functional components are linked together with orange peel and zinc precursors. Organic materials (flavonoids, limonoids, carotenoids) found in orange peel extract also work Ligand agents. These hydroxyl aromatic ring groups are one of extract the components, forming complex bonds with zinc ions. Through the process of nucleation, formation, and nanoparticle formation settled and formed. The nanoparticles were dehydrated using a vacuum machine in the end [1,19].

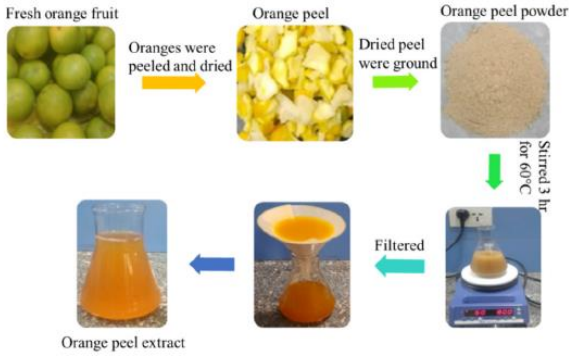

Fig 1. Preparation of green synthesis of orange peel extracts

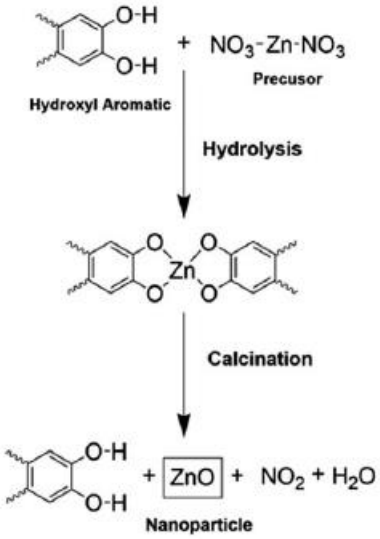

Fig 2. Chemical mechanism of Zinc oxide formation nanoparticles

Graphene Oxide-loaded ZnO nanoparticles

The surface of GO was decorated with ZnO NPs using the previously published techniques with a slight modification[8]. The ZnO NPs (1 mg/ml) were evenly dispersed in 10 ml of de-ionized water and subjected to sonication for 10 minutes to achieve a homogeneous solution. Afterward, the Graphene Oxide was diluted to a concentration of 1mg/mL in 20 mL of de-ionized water and underwent sonication for 10 min in a water bath. The ZnO NPs solution was carefully added to the GO solution while gently stirring and then heated at 50 ◦C for 3 hours. The suspension color undergoes a noticeable transition from a dark yellowish shade to a deep black hue when visually observed. The ZnO/GO composite underwent centrifugation at 12,000 rpm for 12 min. The process was repeated three times to wash the material. Then, the composite was placed in an ice bath for 6 hours and then dried using a vacuum pump.

Electrospinning process:

Preparation of TPU\ZnO@GO NPs nanofibers

To create nanofibers from polymer solutions we use a high electric field (kV), and the pressure and temperature are maintained. Polymeric materials can be electro-spuned to create continuous nanofibers, and several variables affect the properties of the nanofibers. These variables are processing variables (electric field) or polymer properties (viscosity, concentration, and conductivity) and distance from needle tip to collector [20].

The nanofibers of TPU mixing were fabricated via an electrospinning technique [NANON-01, Japan][14]. Here is Table (1) The TPU pellets (10.0 wt%) were dissolved in a solution of Di Methyl Formamide for 6 hours, resulting in a clear and viscous solution. No heating was required for this process. According to Table (1), 10 mL of TPU was added to a glass syringe with a capacity of 20 mL (equipped with an 18G needle), along with different substances derived from ZnO@GO NPs[21]. A high-voltage generator with a power output of 30 kv was used. The direct current generator's positive terminal was connected directly to a collector device, which featured a rotating plate covered in aluminum foil. Figure (3) shows the nanofiber webs were fabricated with meticulous precision using specific parameters: injection of 3 mL/h in a flow rate, an applied voltage of 16 kV, and an electrical charging distance of 12 cm. The humidity and temperature were considered within the electrospinning device[12,13,14].

Table 1: Designed samples and ZnO/GO NPs concentrations.

Code	TPU conc. (Wt.%)	ZnO or ZnO@GONPs (Wt.%)
TPU	10.0	0
TPU\ ZnO	10.0	0.5wt%
TPU\ZnO@GONPs (0.1wt. %)	10.0	0.1wt.%
TPU\ZnO@GONPs (0.3wt. %)	10.0	0.3wt.%
TPU\ZnO@GONPs (0.5wt. %)	10.0	0.5wt.%

Fig.3 TPU@ Graphene oxide/Zinc oxide

Characterization

Scanning Electron Microscope - SEM

Fiber structure was studied using device model DSM-965, the Fiber was gold coated with a sputter coater (Blazers, SCD50). Diameters of Fiber were measured with help of software. In every experiment, Fiber diameter average, and allocation were decided from about 100 random measurements using micrographs that represent Fiber morphology.

Transmission electron microscope- TEM

(TEM) was used to analyze the size, and crystalline structure, by JEOL (TEM-1230, Japan) device.

FTIR

Results were listed by a spectrometer (Nicolet-470Nexus). The FTIR spectrometer was purified constantly with nitrogen. In transmission mode, The infrared spectra were recorded using stout films of spun (solution blow spinning and electrospinning) nanofibers polymer which were represented on a silicon flake.

XRD

For dimensions, nonwoven Fibers were collected on aluminum foil and were stabilized on glass slides for analysis.Scans were done from the angle range(10- 40) (2θ) at a scan rate of two min using CuKα(Ni-filtered)

Anti-bacterial potency

The Anti-bacterial potency of the synthesized TPU and TPU\ZnO@GO NPs was evaluated by conducting tests against *Staphylococcus aureus*
(gram-positive), *Escherichia coli* (gram-negative), [8,15]. The level of inhibition displayed by photos of the plates.

Results and Discussion

Scanning Electron Microscope (SEM)

Table (2) shows figures (a,b,c,d) of the structure of the nanofibers. The Polyurethane nanofiber has a regular and continuous morphology, and the diameter of fibres was in range (140-400 nm)[9].

Transmission electron microscope- TEM

Figure (4) shows the size of Zinc Oxide nanoparticles in the range of (10-20 nm**).**

FTIR

Figure (5) shows the PU sample. NH stretching matches the absorption band at (3323) cm^{-1}. The peaks at (2859-)2938 cm^{-1} are linked with stretching −CH2, and other bands of -CH2 vacillations are identified at (1464-1418-1364-1294) cm^{-1}. Further, a C=O group in polyurethane is linked with the absorption band at (1734) cm^{-1}. Bands of NH group vibrations are identified at (1541) cm^{-1}. hydrogen bonding between N-H and C=O groups at (1702) cm^{-1} is assigned to the (hard/ester) segment or ester-oxygen groups of the soft segments of urethane bonds. nonhydrogen-bonded carbonyl groups band at (1720) cm^{-1} [7,9].

Figure (6) shows that the Polyurethane structure is not affected by the presence of zinc oxide. Electrospun PU shows characteristic bands for (N–H), (C–H), (COO), and (C–C) bonds at (3,320-2,960-1,730-1,703-1,530-1,220) cm^{-1}, respectively. the adsorption of ZnO

Fig (3) FTIR for Polyurethane appeared at the 509 cm^{-1} band. So, the result suggests the union of ZnO in the combined nanofiber[2,3].

The FTIR of TPU@ZnO/GO shows the wavelength for the original O–H (3300–3600) cm^{-1} the sample becomes more visible because the absorption peak, such as wavelength for C = O at (1670-1780) cm^{-1}, C = C (1650–1675) cm^{-1}), C-O at (1050-1150) cm^{-1}, also naturally appeared absorption peaks coupled with some other. wherefore, the analysis of FTIR showed that we successfully converted the graphite into a high purity Graphene Oxide [2,8].

XRD

Figure (7) shows The XRD results, confirmed the presence of ZnO NPs in the dispersed combine. In the original Polyurethane, the spectrum showed no peak, reference to polymer structure nature [10]. While in PU nanofibers doped with the zinc, bands strongly

appeared with maximum peaks at (31.2°-35.8°- 38°) as Bragg's reflection represented from the (100,101,110) planes, which means there are crystalline ZnO. Overall, there was increasing in the crystallinity of the PU polymer because of ZnO.

And at TPU@Zno\GO nanofiber, Graphene Oxide sheets are linked on the surface of nanofibers. like reports in the literature Polyurethane nanofiber film shows a peak around 20° [10]. in the case of the GO/PU nanofiber membrane, the peak of Graphene Oxide was observed at 2θ value of (38°).

More observations for peaks were at 2θ values of (31.2°- 34.3°- 36.4°) identical to the crystal planes (111- 200-220-311).

Further, GO was significantly reduced to RGO indicated by Graphene Oxide peaks indicating that it had been well integrated within the Fiber.

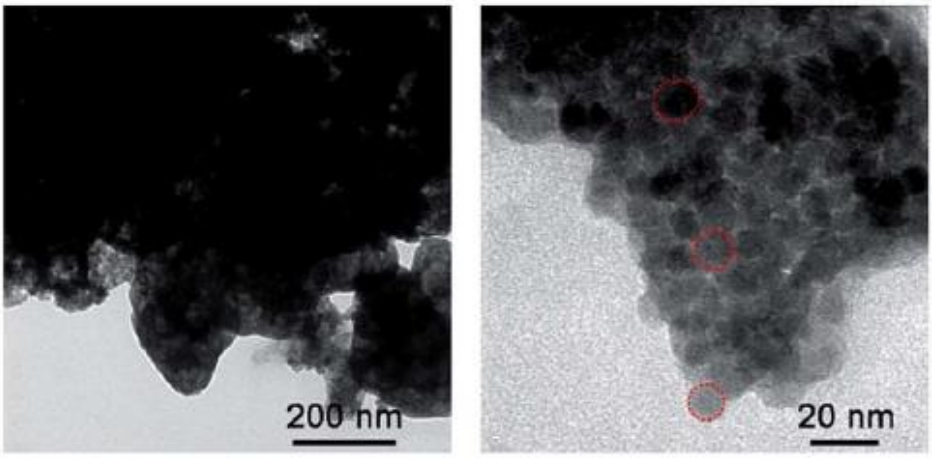

Fig 4. TEM for Zinc Oxide nanoparticles

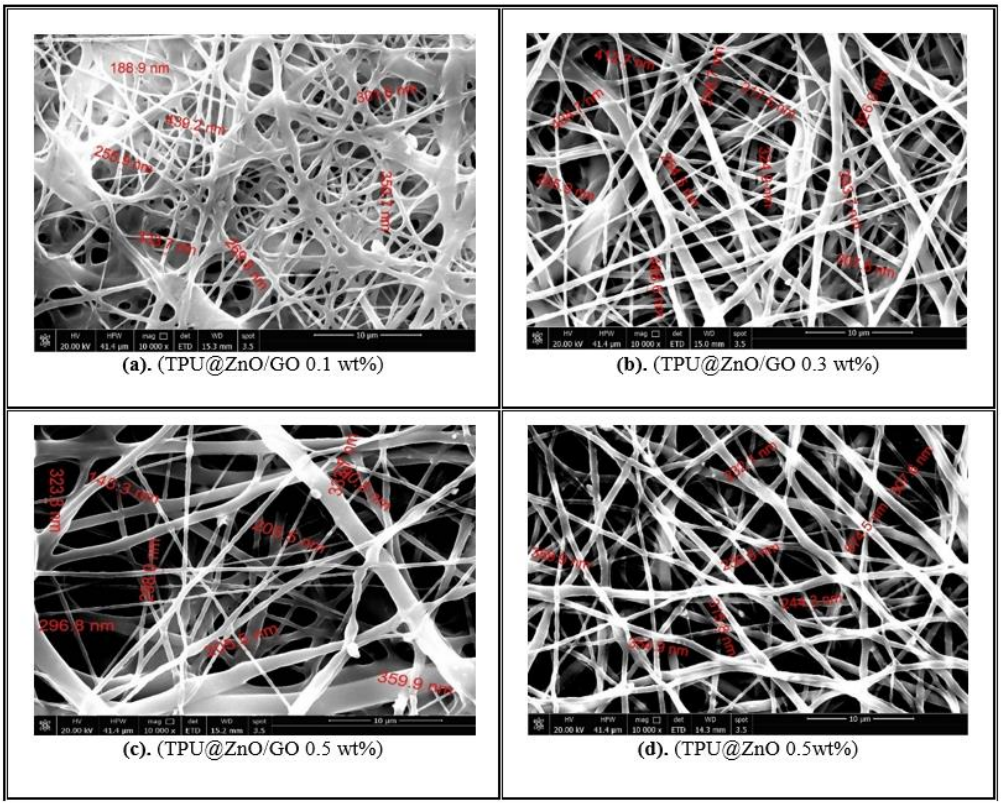

Table 2: SEM of nanofibers with different concentrations of additives

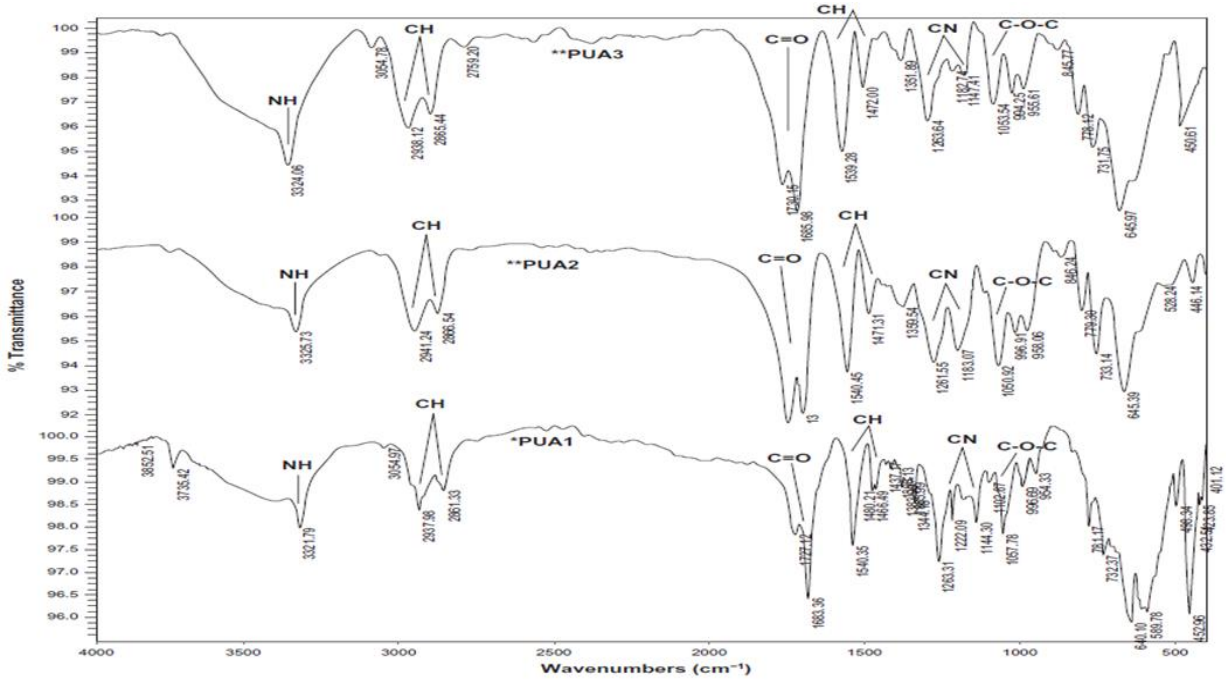

Fig 5. FTIR for Polyurethane (PUA 1,2,3: different polyurethane samples show convergent results).

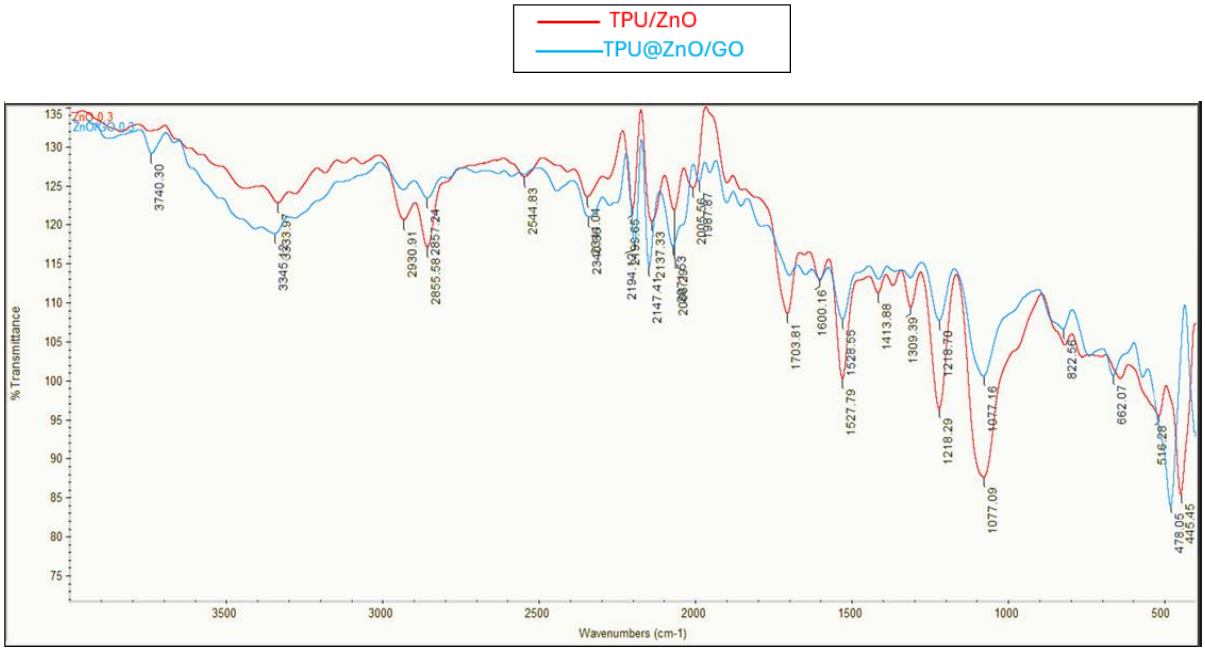

Fig 6. FTIR of TPU@ZnO and TPU@ZnO/GO

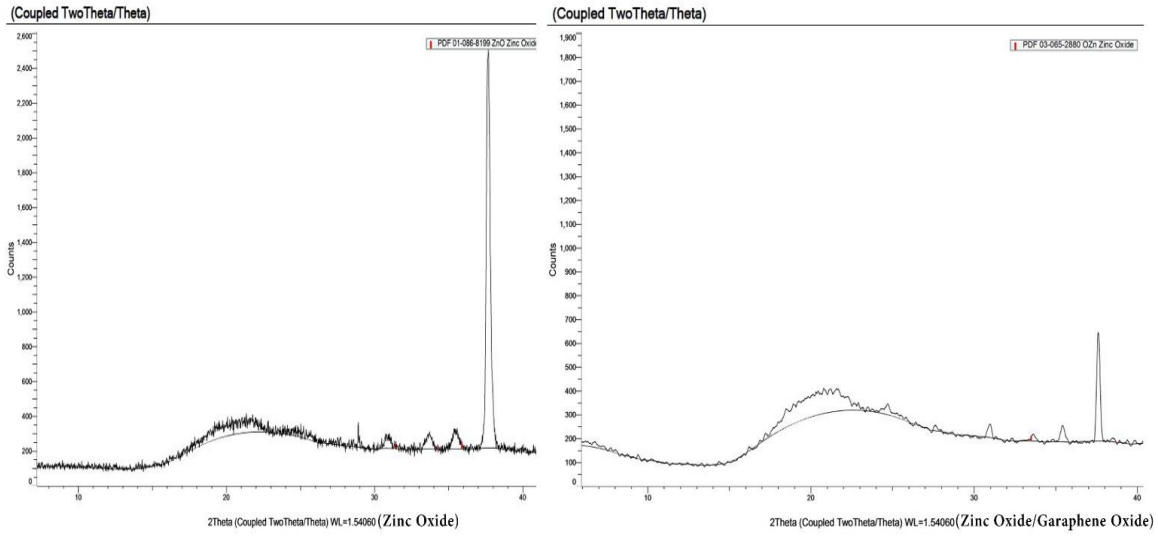

Fig 7. XRD for TPU@ZnO and TPU@ZnO/GO

Anti-bacterial potency:
Methodology

Test strains
Staphylococcus aureus ATCC 6538-P as G+ve bacteria, *Escherichia coli* ATCC 25933 as G-ve bacteria.
The test was assessed by cup agar diffusion procedure.
The nutritional agar was carefully transferred onto Petri dishes using aseptic techniques and subsequently

placed in an incubator for 15 min. Spread a single colony from each bacterial strain over the surface of a Petri dish using a sterile loop in an aseptic manner. The petri dish contained 10 mg of each potential nanofiber.
The dish was placed in an incubator and maintained at a temperature of 37 degrees Celsius for 24 hours. Three experimental replicates were conducted to measure the ZOI (Zone of Inhibition) diameter. In (Table 3) observed no antibacterial activity after treatment TPU with zinc oxide and graphene oxide with *Staphylococcus aureus* G+ve bacteria,
(Table 4) shows the best results in sample (e) (TPU@ZnO/GO 0.5%) the inhibition zone became increasingly clearer and larger with *Escherichia coli* G-ve bacteria.
(Figure 8) shows the effect of ZnO/GO on the shape *E. coli*[4,7].

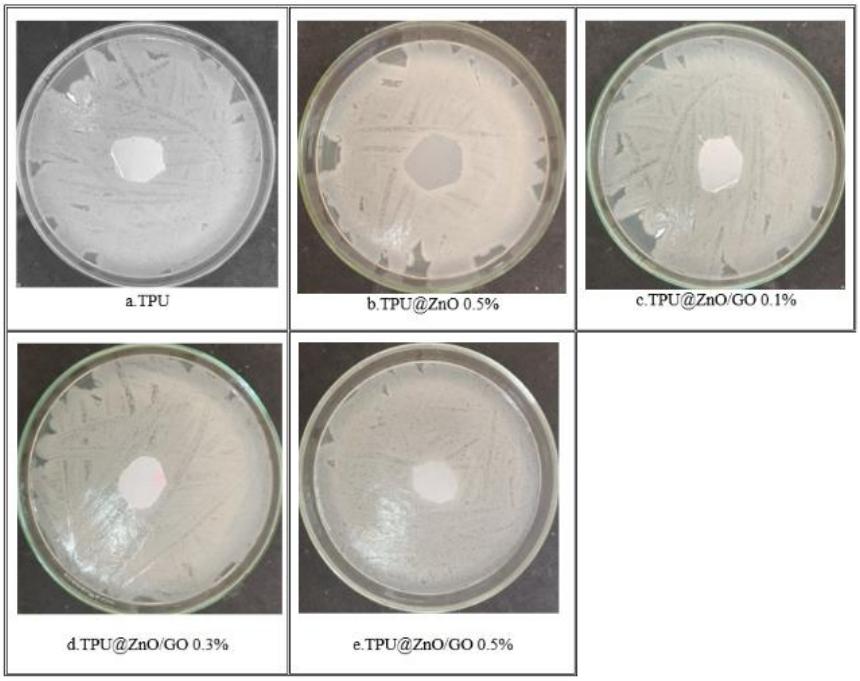

Table (3) Photographs of bacterial colonies formed by Staphylococcus aureus.

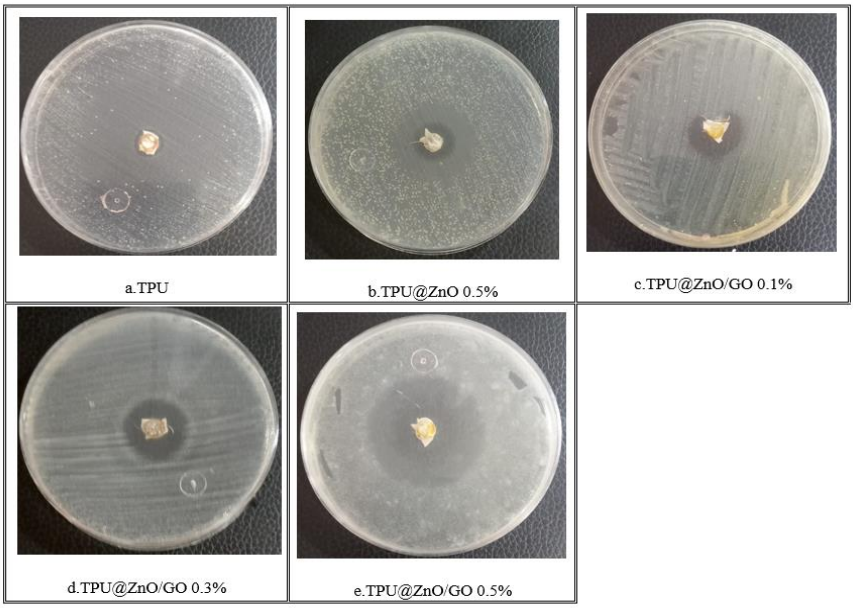

Table (4) Photographs of bacterial colonies formed by *Escherichia coli.*

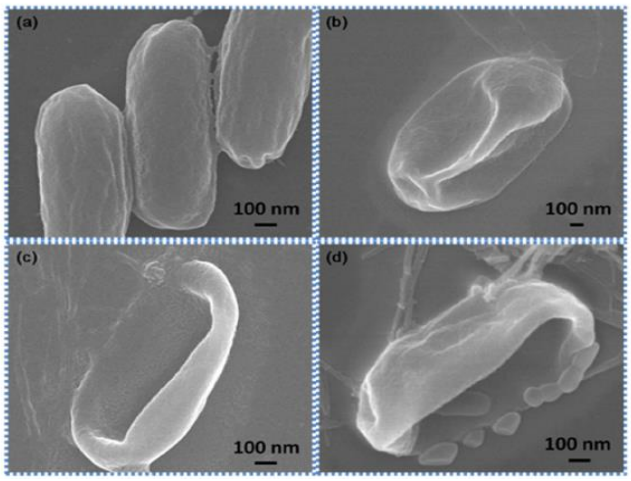

Fig 8. SEM images of bacteria *(E. coli)* treated with TPU@ ZnO/GO. As *E. coli* affected**.**

Conclusions

In this research, ZnO NPs were synthesized by using a green synthesis (Eco-Friendly) method using orange peel extract for zinc oxide as the reducing agent, which reduced using toxic chemicals and cost reduction for the production of nanoparticles. Structural properties of TPU@ZnO/GO nanofiber and nanomaterials were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron microscope (TEM), and X-ray diffraction (XRD), and showed a proven successful result. The composition of ZNO and GO with Polyurethane nanofibers via a one-step electrospinning process was successfully proved. The synthesized nanofiber embedded with ZnO/GO exhibited very strong antibacterial activities against E. coli that showed at the cell membrane and DNA damage.

Conflicts of interest

There are no conflicts to declare.

Formatting of funding sources

No funding sources

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