

# Fabrication and Characterization of Antibacterial Titanium Dioxide Nanorods Incorporating Gellan Gum Films

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## Abstract

The aim of this work was to develop bionanocomposite films based biopolymer GG and TiO<sub>2</sub>-NRs for active food packaging. The TiO<sub>2</sub>-NRs were incorporated as antimicrobials agent into GG via solvent casting method. The films appearance was have 94 % of transparency. SEM micrographs indicate that the TiO<sub>2</sub>-NRs were successfully incorporated and attached to the surface of developed GG films. FTIR results revealed the interaction between TiO<sub>2</sub>-NRs and hydroxyl group of GG polymer. XRD results showed the crystalline peaks of TiO<sub>2</sub>-NRs and amorphous peaks of GG+TiO<sub>2</sub>-NRs films. The thermal stability of GG films were increasing by incorporation of TiO<sub>2</sub>-NRs materials. The GG+TiO<sub>2</sub>-NRs films showed good antibacterial activity against Gram-positive (*Staphylococcus aureus* (*S. aureus*) and *Streptococcus* (strep.)) and Gram-negative (*Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)) and potentially used as antibacterial packaging films.

**Keywords:** gellan gum, TiO<sub>2</sub>-NRs, films, antibacterial.

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## INTRODUCTION

The most favourable biopolymers that are currently used commercially and have been extensive study is gellan gum (GG). GG is an anionic straight chain biopolymer containing  $\beta$ -D-glucose,  $\alpha$ -L-rhamnose and  $\beta$ -D-glucuronate residues<sup>1</sup> and usefulness industrially due to its unique structure and physical properties. Previously, GG has been approached in wound healing study<sup>2,3</sup> as well as candidate material for tissue engineering application<sup>4</sup> and hold a vital role in pharmaceutical. Since 1992, this materials were approved to be utilized in food industry by the US FDA (United States Food and Drug Administration) and the EU (European Union) regulation classified as E 415<sup>5</sup>. GG polymers have been used in varied application included as agar replacement due to its excellent gelling properties as well as highly purified polysaccharide. It has high potential to be apply in industrial as a high-viscosity biogum, a suspending agent and acts as a gelling agent<sup>6</sup>. Not limited to that, GG has been utilized for personal care included in cosmetics and toiletries products<sup>7</sup>. It has thermal stability, clarity and compatibility with nutrient additives<sup>8</sup>. Thus, it's stable at varied temperatures and can offer an excellent stability for varied applications.

TiO<sub>2</sub> nanostructured can be found in four dimension which is 0-D, 1-D, 2-D, and 3-D. It have been create a huge impact on the nanodevices, nanocomposite materials, nanoelectronics, and etc.<sup>9</sup> due to their unique and large surface areas properties. Apart from that, due to the high surface area as well as provided enough absorption sites for all involved molecules in a small space, the TiO<sub>2</sub> nanostructured materials become subject of interest in the research field<sup>10</sup>. Nowadays, the development based on the nanostructured are getting highest ranking over the world either in nanotechnology or nanobiotechnology disciplinary due to their peculiar and fascinating properties. The utilization of TiO<sub>2</sub> nanostructured have been explored widely as a polymers, metals, and ceramics. Their applications not only limit to against human pathogen likes bacteria but also to fight cancer and its was become focal subject in research and applications due to their potent bactericidal activity to against a resistant strains of pathogens. TiO<sub>2</sub> is one of the inorganic materials that have getting special interest to be applied in

inorganic and organic nanocomposites<sup>11</sup>. Among the versatile of TiO<sub>2</sub> nanostructured family, TiO<sub>2</sub>-NRs have become subject of interest and it's typically in 1-D formation and consists of solid elongated structure. Previously, TiO<sub>2</sub>-NRs have been used in development of glucose biosensor<sup>12</sup>, facilitate the DET (direct electron transfer) of GOx (glucose oxidase)<sup>13,14</sup>, in immunotherapy for cancer therapy application<sup>15</sup>, and also as a photoelectrochemical immunosensor<sup>16</sup>. Not limited to that, due to its high extraction proficiency, enhanced for mechanical strength, and outstanding in stability, TiO<sub>2</sub>-NRs display an excellent extraction performance as solid-phase microextraction (SPME) coatings<sup>17</sup>. Above all, TiO<sub>2</sub> is a popular substance in varied application regarding to their properties which is chemically and thermally stable, nature friendly, be biocompatible, cheap, and anti-corrosion.

Therefore, in this study, GG and TiO<sub>2</sub> nanostructured was became an interesting candidate material to be explored as an active food packaging regarding to the environmental concern. The developments of biomaterials as an active food packaging were important in reducing the environmental pollution that causes from accumulation of synthetic packaging. Thus, GG+TiO<sub>2</sub>-NRs films were prepared and characterized using UV, SEM, FTIR, XRD, and TGA and the performance of antibacterial properties also were evaluated.

## EXPERIMENTAL

Prior to the fabrication of film, TiO<sub>2</sub> nanorods was prepared using hydrothermal method<sup>18</sup>. In order to prepare GG incorporating 1wt% TiO<sub>2</sub>-NRs (GG+TiO<sub>2</sub>-NRs) film, 0.01 gram of synthesized TiO<sub>2</sub>-NRs was added into the 100 ml of GG solution. The GG solution was prepare earlier by dissolving 1 gram of GG into 100 ml distilled water. The solution was stirred continuously for 2 hours at 70 °C. Then the homogeneous solution was poured into the casting plate and was dried at 50 °C for 24 h. The films were preconditions for  $\geq 48$  h in the humidity chamber at controlled conditions (relative humidity 50 % at room temperature). The pristine GG films were fabricated using similar technique excluding the addition of TiO<sub>2</sub>-NRs.

Ultraviolet-visible (UV-Vis) transmission was conducted using Varian, Cary 50 spectrophotometer at 200-800 nm of

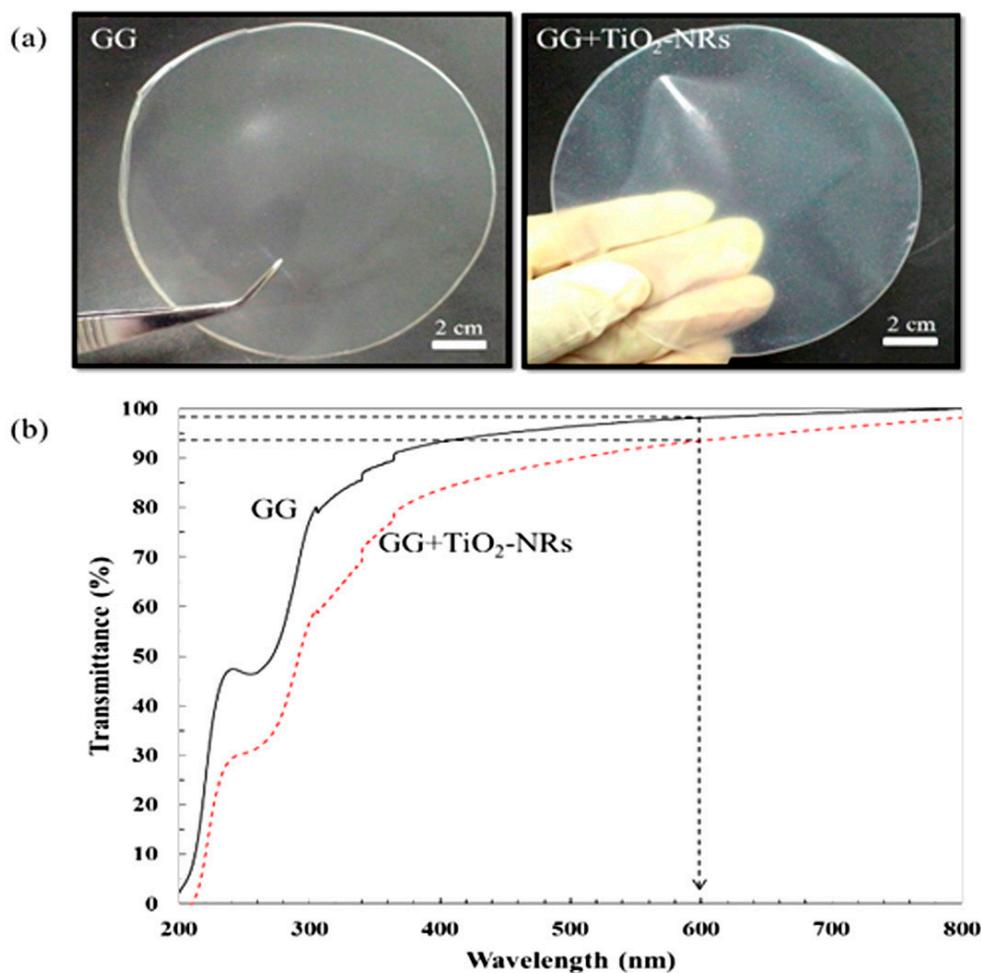
wavelength. Tecnai Biotwin FEI transmission electron microscopy (TEM) was used to observe the TiO<sub>2</sub> nanorods appearance. The morphological images of samples were observed by JOEL JSM 6360 LA electron microscopy. Fourier Transform Infrared Spectroscopy (FTIR) spectra were verified using a Perkin Elmer Spectrum 100 FT-IR spectrophotometer with a PIKE Miracle ATR accessory at 4000 to 600 cm<sup>-1</sup>. XRD analysis was recorded using Rigaku Miniflex (II) X-ray diffractometer from 10° to 80° of 2θ. Thermogravimetric analysis was done using Pyris 6, Perkin-Elmer-TGA6 with heating rate at 10°C/min from room temperature to 1000 °C in an atmosphere of N<sub>2</sub> flow at 50 ml/min.

The antibacterial activity of nanostructured TiO<sub>2</sub> materials were established by

inhibition ring method under UV light irradiation conditions using four types of bacteria which is *S. aureus*, *Strep.*, *E. coli*, and *P. aeruginosa*<sup>19</sup>. At 0.5 McFarland standards, inoculants of each bacteria were evenly spread in sterile petri plates contained the MH agar. Antibacterial properties were evaluated by measuring the transparent antibacterial circle diameter of the materials surrounding bacterial growth after 24 hours.

## RESULTS AND DISCUSSION

Fig. 1a shows the photo images of gellan gum (GG) and gellan gum with the addition of titanium dioxide nanorods (GG+TiO<sub>2</sub>-NRs). The diameter and thickness of both films were almost similar which are ~9 cm and ~60 μm, respectively. The optical transparency of films was evaluated

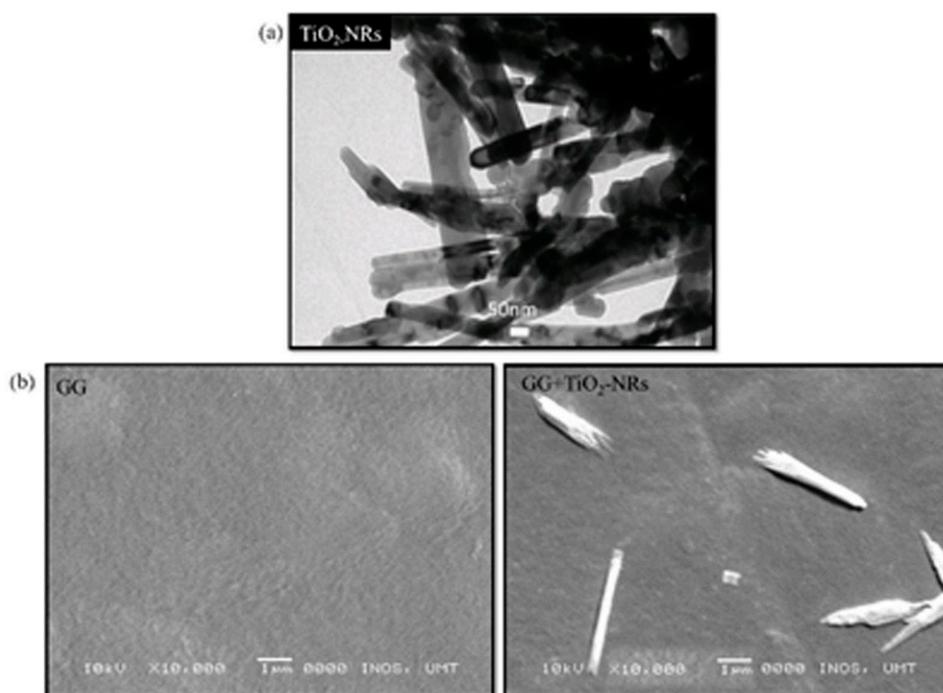


**Fig. 1.** (a) Physical appearance of GG and GG+TiO<sub>2</sub>-NRs (b) UV-Vis transmittance of GG and GG+TiO<sub>2</sub>-NRs films

by UV-visible analysis. The GG and GG+TiO<sub>2</sub>-NRs films were transparent with transmittance values were 98 % and 94 %, respectively (Fig. 1b). Fig. 2a displays the TEM images of TiO<sub>2</sub>-NRs samples. Its exhibits elongated nanostructures (rod-like particles) proposed that the nanorods were successfully obtained. The diameter and length of rod-like particles was found to be 25 – 50 nm and 2.25 – 3.25 μm, respectively. Fig. 2b

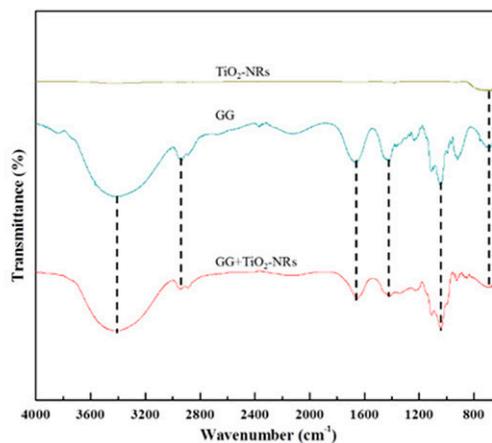
shows smooth and homogeneous surface of GG films. In contrast, less homogenous surface was observed on GG+TiO<sub>2</sub>-NRs films attributed to the agglomeration of TiO<sub>2</sub>-NRs into and onto GG film and contributed to the rough film surfaces that may beneficial for antibacterial activity.

The FTIR spectra of TiO<sub>2</sub>-NRs, GG, and GG+TiO<sub>2</sub>-NRs films are demonstrated in Fig. 3. A broad peak below 1000 cm<sup>-1</sup> has been detected in



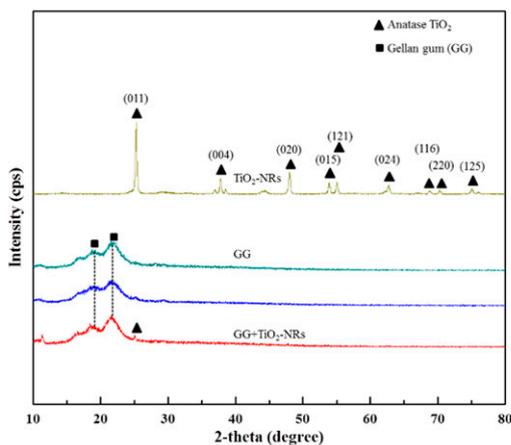
**Fig. 2.** (a) TEM micrographs of TiO<sub>2</sub>-NRs (b) SEM micrographs of GG and GG+TiO<sub>2</sub>-NRs films

the TiO<sub>2</sub>-NRs and GG+TiO<sub>2</sub>-NRs spectrum, which is attributed to metal-oxygen (Ti-O) stretching mode. Apart from that, a broad peak also was display by GG spectra assigned to OH stretching vibrations modes at 3408 cm<sup>-1</sup>, carbonyl group at 1666 cm<sup>-1</sup> and C-C aromatic stretching at 1424 cm<sup>-1</sup><sup>20</sup>. FTIR spectra of GG films also show a prominent peak at 2936 cm<sup>-1</sup> and 1043 cm<sup>-1</sup> due to the C-H stretching modes and glycosidic bonds linking to galacturonic sugar unit in GG chain<sup>21</sup>. Last but not least, another peak was appeared at 692 cm<sup>-1</sup> attributed to the C-H bending mode. Similar peaks were observed in GG+TiO<sub>2</sub>-NRs films as GG, with slightly different in intensity and peak positions due to the interactions among TiO<sub>2</sub> nanostructures and GG biopolymer chain.



**Fig. 3.** FTIR spectra of TiO<sub>2</sub>-NRs, GG, and GG+TiO<sub>2</sub>-NRs films

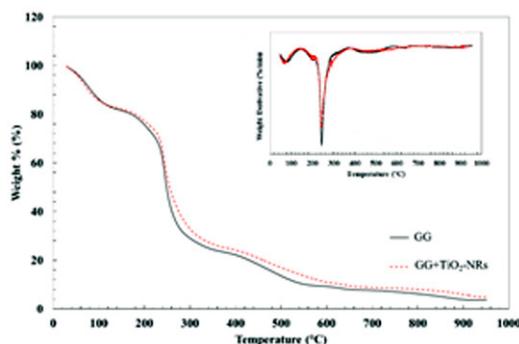
Fig. 4 displays the XRD (X-ray diffraction) diffractogram of  $\text{TiO}_2$ -NRs, GG, and GG+ $\text{TiO}_2$ -NRs films.  $\text{TiO}_2$ -NRs presented sharp peaks at  $25^\circ$ ,  $38^\circ$ ,  $48^\circ$ ,  $54^\circ$ ,  $55^\circ$ ,  $63^\circ$ ,  $69^\circ$ ,  $70^\circ$ , and  $75^\circ$  assigned to (011), (004), (020), (015), (121), (024), (116), (220) and (125) planes of anatase  $\text{TiO}_2$ <sup>22</sup>. The sharp peaks of anatase  $\text{TiO}_2$  proposed that crystalline  $\text{TiO}_2$ -NRs were produced in this study. In contrast, broad peaks which are assigned to amorphous behavior were found at  $18^\circ$  and  $21^\circ$  for both GG and GG+ $\text{TiO}_2$ -NRs films. These peaks were corresponded to the characteristics of GG chains aligned through intermolecular interactions. Additionally, for GG+ $\text{TiO}_2$ -NRs films, one peak with low intensity at  $25^\circ$  was appeared suggesting that  $\text{TiO}_2$ -NRs were successfully incorporated into GG films. This crystalline peak appeared at low intensity due to the small amount of  $\text{TiO}_2$  nanostructures incorporated into GG films and it more apparently appear at high concentration of  $\text{TiO}_2$  nanostructures.



**Fig. 4.** XRD diffractogram of  $\text{TiO}_2$ -NRs, GG, and GG+ $\text{TiO}_2$ -NRs films

Fig. 5 shows the thermogram of GG and GG+ $\text{TiO}_2$ -NRs films. At the 1st stage, the thermal stability shows 18 % of weight loss due to the moisture removal from GG and GG+ $\text{TiO}_2$ -NRs surface. A weight loss at 58 % (GG) and 54 % (GG+ $\text{TiO}_2$ -NRs) in 2nd stage assigned to the depolymerisation and degradation of GG biopolymer chain. A broad decomposition range of GG was observed suggesting to the homogeny distribution of  $\text{TiO}_2$ -NRs in the nano-composites

films. It might be due to the hydrogen bonds formation as well as coordinate interaction between  $\text{TiO}_2$  and GG. Addition of  $\text{TiO}_2$ -NRs into GG increased the temperature to end up the degradation step regarding to the final temperature were verified at  $390^\circ\text{C}$  for GG+ $\text{TiO}_2$ -NRs films showed it contains higher heat resistant. Addition of  $\text{TiO}_2$ -NRs exhibits better outcome in thermal stability regarding to the certain degree of interaction between GG and  $\text{TiO}_2$ -NRs as revealed



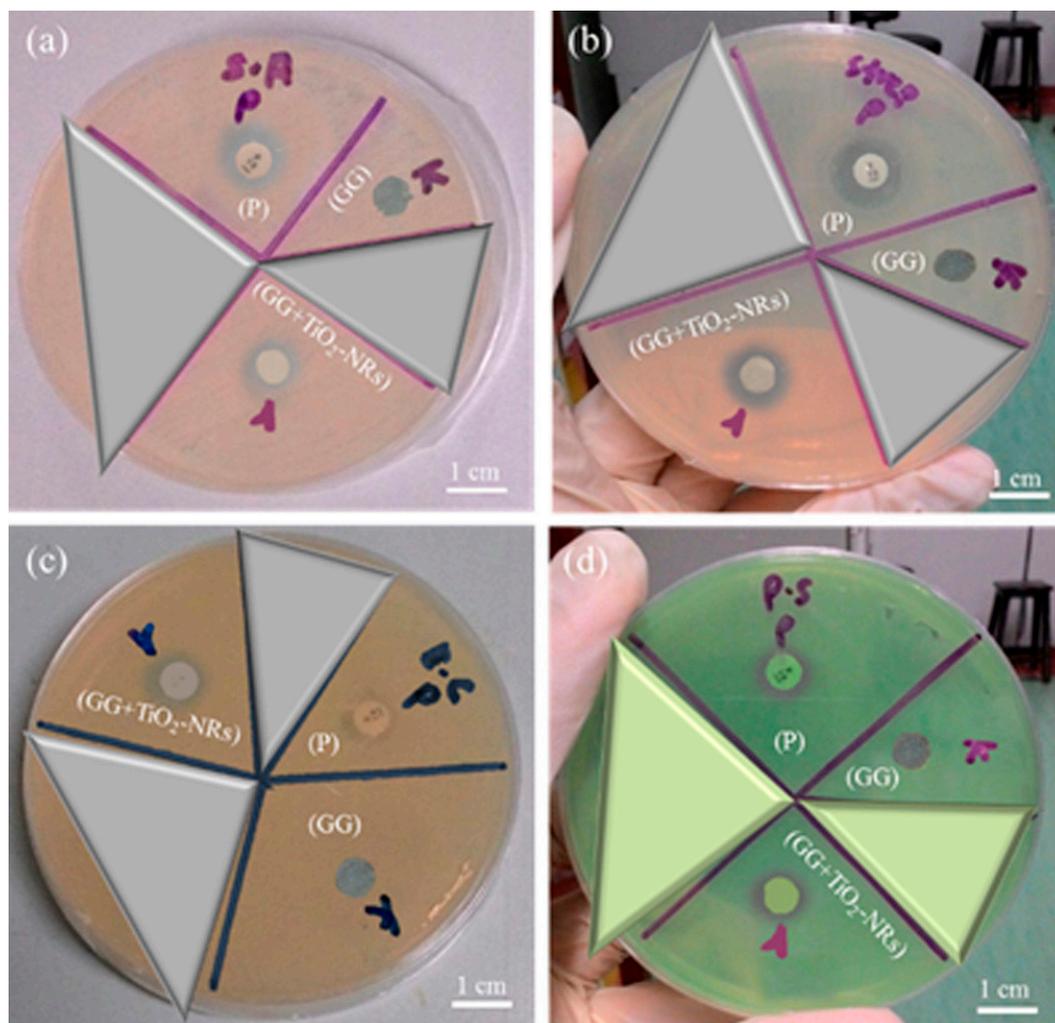
**Fig. 5.** TGA thermogram of GG and GG+ $\text{TiO}_2$ -NRs films

by FTIR spectroscopy.

Fig. 6 shows no antibacterial activities were found after 24 h incubation of pure GG film for all the bacteria. However, the inhibition zone was recorded using GG+ $\text{TiO}_2$ -NRs films at  $10 \pm 0.35$  mm,  $12 \pm 0.06$  mm,  $11 \pm 0.06$  mm, and  $10 \pm 0.38$  mm against *S. aureus*, *Strep.*, *E. coli*, and *P. aeruginosa* which is comparable with penicillin control sample (Table 1). Good antibacterial activity by GG+ $\text{TiO}_2$ -NRs films was due to the presence of  $\text{TiO}_2$  nanostructures which is promoted the ROS (reactive oxygen species) generation, release of metal ion, malfunction of membrane system, and internalization of nanoparticles<sup>23</sup>. With the present of UV light, the  $\text{TiO}_2$ -NRs that were incorporated into GG films produced ROS species (eg.  $\text{HOO}\cdot$ ,  $\text{HO}\cdot$ , and  $\cdot\text{O}_2^-$ ) from  $\text{O}_2$  and  $\text{H}_2\text{O}$  through reduction reaction. The electron in the  $\text{TiO}_2$  conduction band will leave a positive hole in the valence band when the  $\text{TiO}_2$ -NRs are irradiated by UV. Then, the photo-generated electron will react with molecular oxygen as well as with water to produce superoxide radical anions ( $\cdot\text{O}_2^-$ ) and hydroxyl ( $\cdot\text{OH}$ ) radicals, respectively. Therefore, these reactive radicals will executed the bacteria by decomposed the

organic compounds in bacterial cells<sup>24-28</sup>. The result obtained suggesting that, TiO<sub>2</sub>-NRs is a promising

antibacterial agent as GG+TiO<sub>2</sub>-NRs films exhibited excellent antimicrobial property.



**Fig. 6.** Antimicrobial activity of GG and GG+TiO<sub>2</sub>-NRs films against (a) *Staphylococcus aureus*, (b) *Streptococcus*, (c) *Escherichia coli*, and (d) *Pseudomonas aeruginosa*

**Table 1.** Inhibition zone of films against *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli*, and *Pseudomonas aeruginosa*

Sample/ Inhibition Diameter (mm)	<i>S. aureus</i>	<i>Strep.</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Penicillin	12±0.06	12±0.06	10±0.12	9±0.06
GG	-	-	-	-
GG+TiO <sub>2</sub> -NRs	10±0.35	12±0.06	11±0.06	10±0.38

(Data presented are mean±SD, n=3)

## CONCLUSION

GG+TiO<sub>2</sub>-NRs film was successfully developed by solvent casting technique. The transparency of films was reduced from 98 % to 94 % when the TiO<sub>2</sub>-NRs were added into GG films. FTIR, XRD, and TGA results obtained exhibited the interaction of TiO<sub>2</sub> nanorods with the GG polymer chain. SEM micrograph shows the presence of TiO<sub>2</sub> nanorods on the surface of film and were enhanced the antibacterial activity.

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

## FUNDING

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## AUTHOR'S CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

## ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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