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Synthesis and Antibacterial Efficacy of Nipa Palm Vinegar-Graphene Quantum Dots against *Staphylococcus aureus* and *Escherichia coli*

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Nipa palm vinegar (NPV) is an essential product from the Pak Phanang district in the Nakhon Si Thammarat Province, Thailand. NPV is used for cooking and its antibacterial activity reduces dependency on toxic chemicals such as antibiotics and preservative foods. Using NPV as the precursor, nipa palm vinegar–graphene quantum dots (NPV-GQDs) were successfully synthesized through pyrolysis. Preliminary screening of the antibacterial activities of NPV and NPV-GQDs against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains were determined by using the agar well diffusion method. The broth macro-dilution method determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The diameter of the inhibition zones of *S. aureus* and *E. coli* reached 29.30 mm and 23.50 mm, respectively. The MIC of NPV-GQDs against *S. aureus* and *E. coli* was 6.25 mg/mL and 12.50 mg/mL, respectively, whereas the MBC of NPV-GQDs against *S. aureus* and *E. coli* was 50.00 mg/mL. These results signify the potential healthcare application of NPV-GQDs as high-quality and valuable antimicrobial agents, thereby reducing chemical usage toxic to the body in the future.

Keywords: Nipa palm vinegar, Graphene quantum dots, Antibacterial activity.

INTRODUCTION

Graphene quantum dots (GQDs) are a novel class of materials with significant interest among researchers worldwide in recent years and are composed of a few layers of less than 10 nm graphene fragments and absorb light in the UV range (230-300 nm) [1]. These nanoparticles are chemically and physically stable, have a high specific surface area and rapidly disperse in water due to functional groups at the edges [2]. As a result, the fluorescence emission of GQDs can span a wide spectral range, including UV, visible and infrared. The origin of fluorescence emission when GQDs are disseminated in water has been linked to quantum confinement effects, defect states and functional groups being affected by pH [3]. In addition, the crystallographic orientation of GQD edges has a strong influence on their electrical structure *e.g.* zigzag-edge GQDs with diameters ranging between 7-8 nm exhibit metallic properties [4]. Due to their unique properties, GQDs have been used in bioimaging, cancer therapies [5], temperature sensing [6], drug delivery [7], LED lighter converters, photodetectors, OPV solar cells, photoluminescent materials and biosensor fabrication [8].

Recently, GQD_S and their composites have been involved in antibacterial applications. Teymourinia *et al.* [9] reported that Cu_2O/GQDs showed the highest antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Mydeen

et al. [10] found that GQD/ZnO displayed enhanced antibacterial activity against *P. aeruginosa*, even at low concentrations. Li *et al.* [11] reported that GQDs enhanced the antibacterial efficiency of nano-ZnO/GQDs hydrophobic to 85.7%. Rashki *et al.* [12] discovered that ZnO/GQDs nanocomposites grafted from the extract of *Protoparmeliopsis muralis* could inhibit bacterial growth (methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE)). Similarly, Teymourinia *et al.* [13] prepared the TiO_2/Sb_2S_3/GQDs nanocomposites as efficient antibacterial agents against *E. coli* and *S. aureus*. Thus, GQDs and their composites have been identified as potential antibacterial agents.

GQDs are prepared using different carbon precursors like carbon nanotubes (CNTs), graphene oxide (GO), carbon black [14], shallot extract [15] and citric acid [16,17]. Furthermore, certain fruit juices are also suitable precursors of GQD synthesis due to the low carbonization temperature. Moreover, fruit juices are sour due to acids, such as citric acid [18,19]. This study reported for the first time an ecofriendly green pyrolysis synthesis approach to develop graphene quantum dots (NPV-GQDs) utilizing Nipa palm vinegar from the fermented juice as precursor. Scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and ultraviolet-visible spectroscopy (UV-Vis) were used to examine the synthesized NPV-GQDs. In addition, the antibacterial activity of NPV-GQDs was tested using the agar well diffusion method against *E. coli* and *S. aureus* strains.

EXPERIMENTAL

Nipa palm vinegar-graphene quantum dots (NPV-GQDs) preparation: All samples of nipa palm vinegar (NPV) was collected from the Pak Phanang district in Nakhon Si Thammarat province, Thailand. Then, Nipa palm vinegar (NPV) was used as the precursor for developing NPV-GQDs by the pyrolysis method. First, approximately 200 mL of NPV was added into a beaker. Then, the beaker was heated to 200 ºC for about 30 min using a paraffin oil bath. Initially, the liquid sample was colourless; then, it changed to orange after NPV-GQD synthesis. Next, the reaction was stopped using 0.25 mol/L NaOH, with continuous stirring for 30 min. Then, the NPV-GQDs were cooled at room temperature and kept in the fridge before use. Finally, the formation of NPV-GQDs was confirmed by using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and ultraviolet-visible spectroscopy (UV-Vis) techniques.

Antibacterial activity test: The antibacterial activity of NPV-GQDs against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial strains were evaluated. The bacterial strains were obtained from the Science Center of Nakhon Si Thammarat Rajabhat University, Thailand. The antibacterial activity of the NPV-GQDs was tested using the agar well diffusion method. Fresh cultures of bacterial strains cultivated on sterile Muller Hinton Agar (MHA) were used to make the inoculum. A loopful of bacterial culture was inoculated into a Muller-Hinton Broth medium and incubated at 37 ºC for 24 h. The culture size was set to 0.5 McFarland standard turbidity, which equaled about 10^8 colony-forming units (CFU/mL). Using a sterile wire loop, cell suspensions (100 mL of target strain) were put into MHA plates and dispersed thinly on the plates. After the medium had set, 8 mm diameter disks were cut out of the agar and 100 mL of NPV-GQDs or citric acid-graphene quantum dots (CA-GQDs) as a control were deposited in each well. The plates were then incubated for 24 h at 37 ºC. After 24 h, the widths of the inhibition zones around the well (in millimeters) were measured.

Determination of MIC and MBC: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using a modification of the dilution tube method. Briefly, in eight sterile test tubes, 1 mL Muller Hinton Broth (MHB) was added. Then, in the first test tube, 100 mg/mL of dissolved NPV-GQDs was added, mixed thoroughly and 1 mL was transferred from the first test tube to the second test tube and then to the third test tube. This was repeated until the eighth test tube, after which 1 mL of the content was removed and discarded. A 0.5 McFarland standard 1 mL of *E. coli* culture was injected into each test tube. After ensuring that the negative control tube revealed no growth, the method was repeated for *S. aureus*, mixed well and incubated at 37 ºC for 18-24 h. Then, a loopful of broth from each test tube not showing growth was inoculated into an MHA plate and incubated further for 24 h at 37 ºC. The tubes and agar plates were examined for the growth of the bacteria.

RESULTS AND DISCUSSION

FT-IR studies of NPV-GQDs: The NPV-GQDs samples were characterized by FTIR spectroscopy. A clear peak was observed at 1590 cm^{-1}, corresponding to the vibration of C =C bonds, while a broad vibration at roughly 3430 cm^{-1} depicted the vibration of O–H bonds (Fig. 1). The hydroxyl of the acquired NPV-GQDs was primarily attributed to the O–H peak, possibly corroborating the high vibration of C-O at about 1270 cm^{-1}. The C–H vibration was responsible for the prominent peak at about 870 cm^{-1} [20]. Fig. 2 showed the UV-Vis spectra of NPV-GQDs, which had absorption peaks at 278 nm and 350 nm, ascribed to the $\pi \rightarrow \pi^*$ of C = C and $n \rightarrow \pi^*$ transition of C=O, respectively, as reported for GQD-based materials [18].

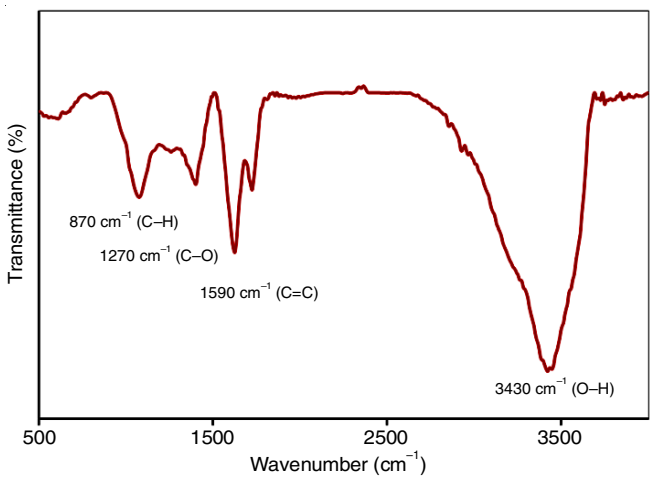

Fig. 1. FTIR spectra of NPV-GQDs

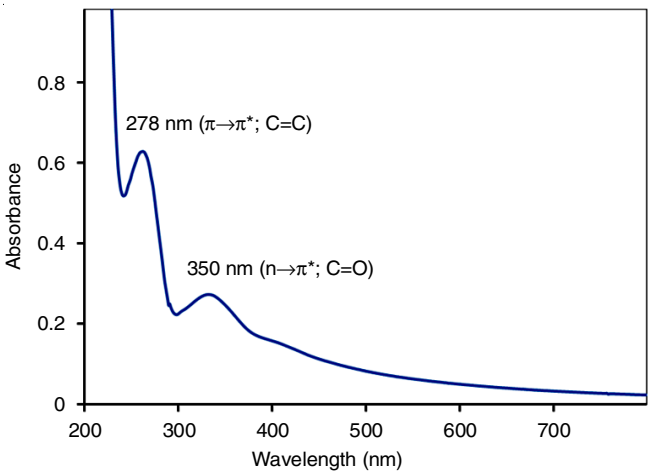

Fig. 2. UV-vis spectra of NPV-GQDs

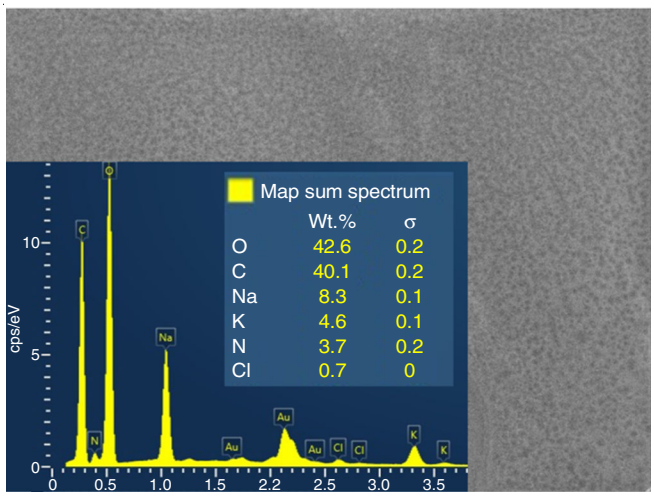

Fig. 3. SEM image and EDX spectrum of NPV-GQDs

The morphology of NPV-GQDs was evaluated by SEM analysis (SEM, Oxford, UK). Fig. 3 demonstrates that synthesized NPV-GQDs were spherical with particle sizes in the nanometer range. The chemical composition and purity of the synthesized NPV-GQDs were analyzed using EDX (EDX, Oxford, UK). The EDX spectra showed various elements, including O, C, Na, K, N and Cl, which accounted for 42.6%, 40.1%, 8.3%, 4.6%, 3.7% and 0.7%, respectively. Moreover, all the elements were distributed properly (Fig. 4).

Antibacterial activity of NPV-GQDs: The synthesized NPV-GQDs exhibited significant antibacterial activities against *S. aureus* and *E. coli* with inhibition zones of 29.30 nm and 23.50 nm, respectively. However, the inhibition zone of NPV against both *S. aureus* and *E. coli* were noted as 20.00 nm (Figs. 5 & 6 and Table-1). It was observed that NPV-GQDs had higher antibacterial activity than NPV. Due to the smaller particle size and larger surface area, NPV-GQDs inhibited bacterial strains better than NPV. In addition, it was clear that *S. aureus* had higher sensitivity than *E. coli*. Therefore, the NPV-GQDs could destroy the cell structures of *S. aureus* better than *E. coli*.

TABLE-1
ZONE OF INHIBITION OF NPV-GQDs
COMPARED TO NPV AND CA-GQDs

Type of active material	Inhibition zone (mm)	
	S. aureus	*E. coli*
NPV	20.00	20.00
NPV-GQDs	29.30	23.50
CA-GQDs	25.70	21.00

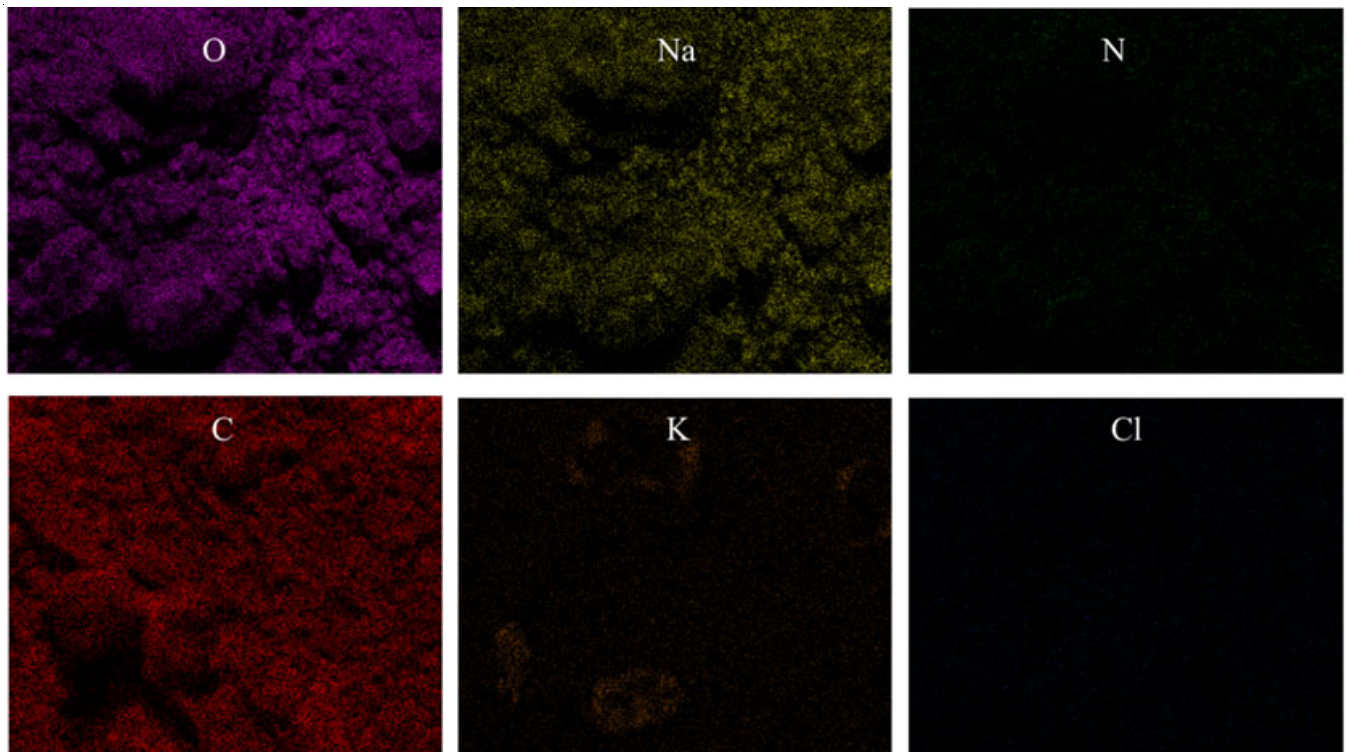

Fig. 4. EDX mapping analysis of NPV-GQDs

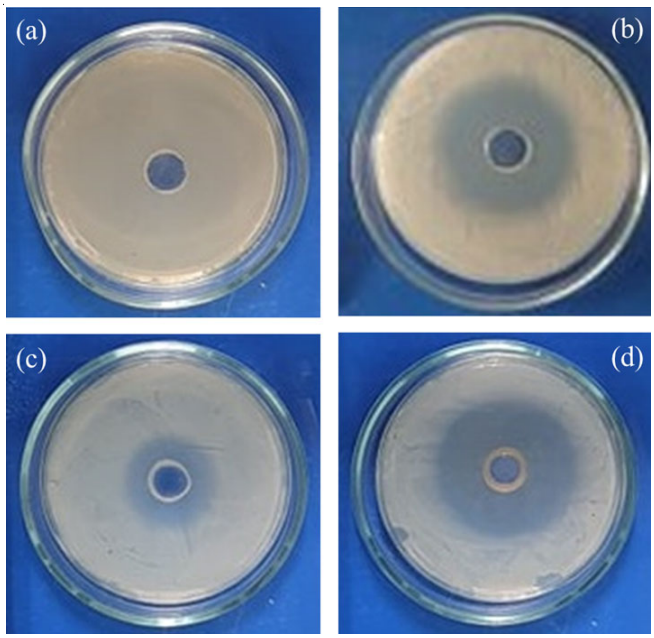

Fig. 5. Inhibition zone of (a) NaOH (negative control), (b) CA-GQDs (positive control), (c) NPV and (d) NPV-GQDs for *S. aureus*

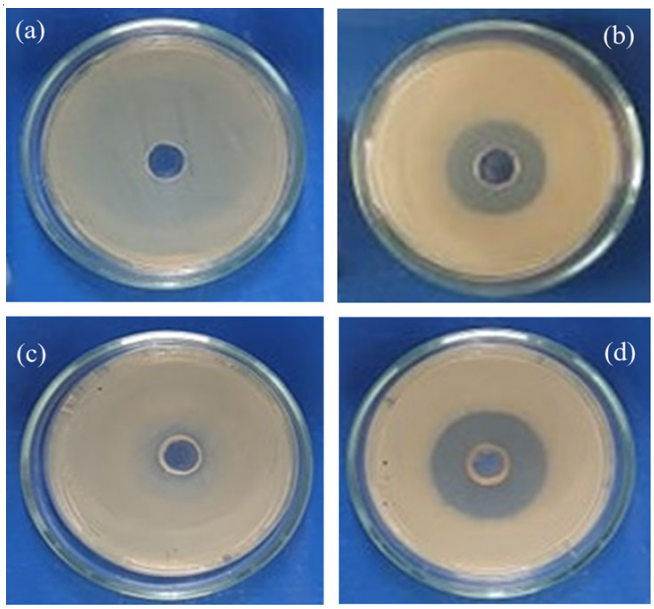

Fig. 6. Inhibition zone of (a) NaOH (negative control), (b) CA-GQDs (positive control), (c) NPV and (d) NPV-GQDs for *E. coli*

Antibacterial activity mechanisms of NPV-GQDs: The NPV-GQDs could have interacted with the cell membranes of *S. aureus* better than *E. coli* due to different bacterial cell walls and membrane structures, which could explain the antibacterial mechanism. Generally, Gram-positive bacteria are relatively easier to counteract than Gram-negative bacteria. Gram-positive and Gram-negative bacteria have different chemical compositions of their cell walls. So, the entry of NPV-GQDs in the bacterial cell led to the change in DNA molecule into condensed form and caused cell death. Moreover, the NPV-GQDs are also involved in producing reactive oxygen species (ROS) [19]. ROS promotes cellular oxidative damage and causes cellular injury.

Furthermore, the impact of the toxicity of NPV-GQDs on bacterial inhibition was further assessed by determining the MIC. The MIC of NPV-GQDs through the broth dilution method against *S. aureus* and *E. coli* were 6.25 and 12.5 mg/mL (Table-2), respectively. The MIC values against *S. aureus* and *E. coli* could be influenced by the large surface area and high penetrating power of NPV-GQDs, depicting enhanced interaction with microorganisms and greater lethality [21]. However, both NPV and NPV-GQDs could kill the bacterias at a concentration of 50 mg/mL.

TABLE-2
MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF NPV-GQDs AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Type of active material	Gram-positive (*S. aureus*)		Gram-negative (*E. coli*)	
	MIC	MBC	MIC	MBC
NPV-GQDs concentration (mg/mL)	6.25	50.00	12.50	50.00

Conclusion

The current study reported the Nipa palm vinegar (NPV) to synthesize NPV-GQDs using a green synthesis approach. Multiple approaches were used to confirm the development of the NPV-GQDs. The synthesized NPV-GQDs had outstanding antibacterial efficacy, with MIC of 6.25 mg/mL against *E. coli* and 12.5 mg/mL against *S. aureus*. Moreover, the MBC of NPV-GQDs was 50.00 mg/mL against *E. coli* and 50.00 mg/mL against *S. aureus*. Thus, the NPV-GQDs possess the significant potential as high-quality antibacterial materials in the healthcare sector.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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