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STUDY ON THE ANTIBACTERIAL ABILITY OF THE HYBRID MATERIAL BETWEEN PORPHYRIN AND NANO SILVER

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Abstract. Water-soluble porphyrins di(4-aminophenyl)-di(4-sulfophenyl)-diphenyl-porphyrin $[(SO_3)_2\text{-TPP-}(NH_2)_2]$(TASP) and nano-silver materials (AgNPs) were successfully synthesized by chemical synthesis and green synthesis from guava leaf extracts, respectively. The silver nanoparticle size is well controlled in the range of 1.1 - 1.5 nm. Then, AgNPs/TASP hybrid materials were successfully synthesized by different mixing methods. The UV-Vis spectrum of the hybrid material depends on the AgNPs:TASP mixing ratio and still shows the Soret peaks and Q-bands characteristic of the porphyrin family and the broad absorption range from 300 - 600 nm of the silver nanoparticles initial. The results of an antibacterial test against both gram-negative and gram-positive strains on LB agar showed that all samples had different antibacterial activities. In which, the AgNPs/TASP hybrid material has better inactivation ability than silver nano for both tested bacterial strains. Besides, the presence of polypyrrole conducting polymer (PPy) has promoted the kinetics of silver ion release, which also gives outstanding antibacterial results, thereby opening the potential to fabricate hybrid materials for antibacterial applications.

Keywords*:* porphyrin, water soluble porphyrin, silver nanoparticles, antibacterial, polypyrrole.

1. Introduction

Silver nanomaterials are of interest to researchers because of their special properties, such as optical properties with surface plasmons and bactericidal properties [1-5]. Silver nanoparticles (AgNPs) are mostly fabricated by chemical methods, due to many

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transformation reactions, synthesis efficiency and purity are not high. Therefore, it may affect the bactericidal and virus-killing ability of nano silver as well as its toxicity due to accumulation of Ag^+. The current research trend to create silver nanoparticles is gradually turning to a green synthesis method: using extracts from plants as reducing agents. This method is simple, environmentally friendly, and without harmful chemicals and dangerous by-products [6, 7].

Porphyrin family materials and their derivatives have also been studied and proven to have many biomedical roles thanks to their superior properties, low toxicity, biocompatibility, and physical, chemical, and biological unique properties such as applications in biodiagnostics, biomedical markers, biological imaging, antibacterial, cancer treatment by photodynamic and photothermal therapies [8]. The antibacterial and antiviral activities of the porphyrins are based on their ability to catalyze peroxidase and oxidase reactions, absorb photons and generate reactive oxygen species (ROS) and cleave the lipid component of bacterial membranes. The antibacterial activity of porphyrins depends on many factors such as composition, structure, photodynamic activity, and light excitation [9-13].

Nanosilver and porphyrin hybrid materials have also been studied and proven to increase efficiency, kill bacteria, as well as have a broader antibacterial spectrum than the individual materials [14-16]. Most of the porphyrins with non-polar substituents such as alkyl, aryl... are insoluble or difficult to dissolve in water, but when adding highly polar or charged functional groups such as SO_3^{2}-, COO^-, SO_3Na^-... porphyrins can dissolve in water and easily interact, encapsulate and stabilize Ag nanoparticles in polar solutions such as water and alcohol [14, 15, 17]. Although there have been some published reports on the antibacterial ability of porphyrin when combined with nano silver, there have not been any studies on the antibacterial ability of water-soluble porphyrin di(4-aminophenyl)-di(4-sulfophenyl)-diphenyl-porphyrin $[(SO_3)_2$-TPP-$(NH_2)_2]$ (TASP) when combined with silver nanoparticles was synthesized by biological methods [14, 15]. This is the first study on creating AgNPs/TASP hybrid materials and testing their antibacterial ability. Investigation of the extract of guava leaves has shown that the water-soluble components of the extract such as tannins, eugenol, and flavonoids can participate in the reduction of Ag^+ to Ag^0 [18]. The objective of this study is to create a safe, environmentally, and human-friendly hybrid nanomaterial based on AgNPs and porphyrin high antibacterial activity.

2. Content

2.1. Materials and methods

**** Chemicals***

Chemicals used to synthesize TASP include tetraphenylporphyrin (H_2TPP), purity 96%; HNO_3 of Xilong, China 98%; sodium sulfide nonahydrate ($Na_2S\ 9H_2O$) of Korea, purity ≥ 98.0%; N,N- Dimethylformamide (DMF) of Xilong, China, purity 98%; sulphuric acid (H_2SO_4) of Xilong, China, purity 98%; sodium hydroxide (NaOH) of Xilong, China, purity 98%.

Chemicals used to synthesize silver nanoparticle (AgNPs) include fresh guava leaves; silver nitrate ($AgNO_3$) of Xilong, China with 98% purity; isopropyl alcohol of Merck with 99.8% purity; and double distilled water.

Chemicals used to synthesize polypyrrole include pyrrole (C_4H_5N) of Sigma-Aldrich with 98% purity; $FeCl_3$ of Xilong, China with a purity of 98%.

All chemicals and reagents were of analytical grade and used without any further purification.

**** AgNPs synthesis from guava leaf extract***

20 g of fresh guava leaves are washed, ground into powder, and boiled with 100 mL of distilled water for 30 minutes. The extract was then filtered and centrifuged at 5000 rpm for 30 min to remove impurities. Then slowly add 0.01 M silver nitrate to the extract according to the volume ratio $V_{extract}:V_{Ag+}$ = 1:9 at room temperature [19] and stir with a magnetic stirrer for 2 hours to obtain a solution of AgNPs (Figure 1).

Figure 1. Schematic of synthesis of AgNPs by green synthesis method

**** Synthesis of water-soluble porphyrin [$(SO_3)_2$-TPP-$(NH_2)_2$] (TASP)***

Water-soluble porphyrin (TASP) was synthesized according to the method of Cui, X. et al., 2019 (Figure 2) [20].

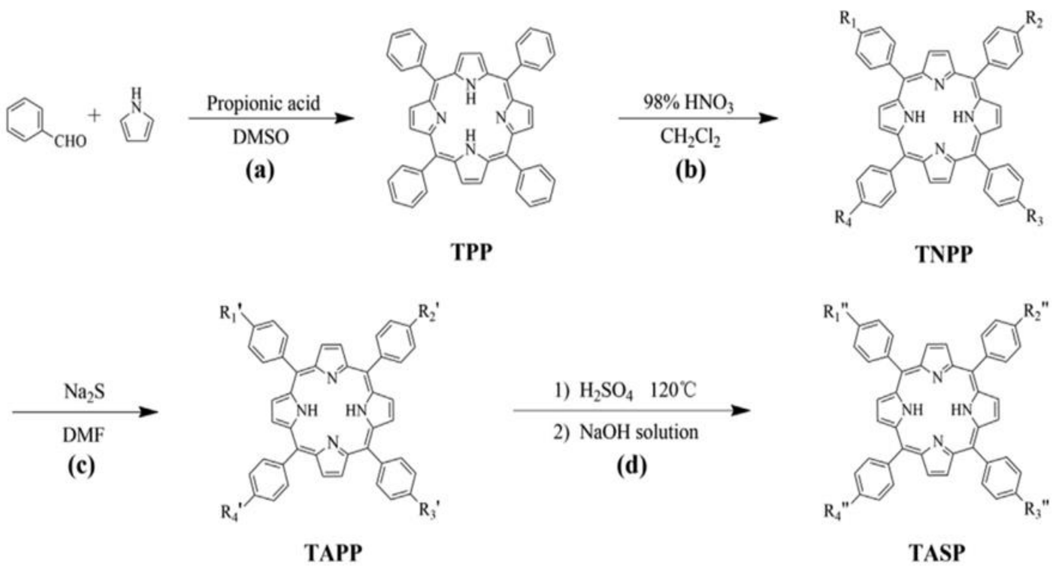

Figure 2. Schematic diagram of the synthesis of di(4-aminophenyl)-di(4-sulfophenyl)-diphenyl-porphyrin [$(SO_3)_2$-TPP-$(NH_2)_2$] (TASP) [20]

**** Synthesis of polypyrrole conducting polymer (PPy)***

Polypyrrole conducting polymer (PPy) was synthesized according to the method of Singh, A. et al., 2013 [21].

Slowly add the pyrrole solution to the iron salt solution with magnetic stirring to obtain a homogeneous mixture. This mixture will turn from transparent to yellow and then dark black. The black color of the mixture indicates that the reaction has formed polypyrrole. Filter and vacuum to collect polypyrrole in powder form.

**** Synthesis of AgNPs/TASP hybrid materials***

1 mL of guava leaf was extracted and mixed with 4 mL of TASP dissolved in water ($2.35.10^{-5}$ M). Add $AgNO_3$ solution (0.01 M) slowly to the mixture of extract and TASP. Centrifuge the mixture at 5000 rpm for 30 min to collect hybrid material between silver nanoparticles and water-soluble porphyrins (AgNPs/TASP).

**** Synthesis of AgNPs/TASP + polypyrrole (PPy) hybrid materials***

Polypyrrole (0.02 mg) powder was dispersed in 10 mL of AgNPs/TASP and dissolved by ultrasonic vibration to obtain AgNPs/TASP + polypyrrole (PPy) hybrid material at room temperature.

**** Investigate the UV-Vis absorption properties of the hybrid materials***

UV-Vis measurement was performed using a Biochrom spectrometer at the Vietnam Academy of Science and Technology in the spectral range from 230 to 800 nm. Solutions of samples were prepared in quartz curves using suitable solvents.

**** Determine the particle size distribution by dynamic light scattering DLS***

Particle size measurements by DLS dynamic light scattering were performed on a HORIBA LB-550 instrument. The sample solutions were added to a quartz curve and placed into the sample chamber for measurements.

**** Test the antibacterial ability of the materials***

Escherichia coli (Gram -) and Staphylococcus aureus (Gram +) were used as control strains to evaluate the antibacterial ability of the synthesized materials. All samples (in Table 1) were tested in triplicate.

Table 1. Concentrations of materials used in antimicrobial testing

Materials	**Concentration**
AgNPs	0.01 M*
AgNPs/TASP	0.01 M*
PPy	0.004 mg/mL
AgNPs/TASP+PPy	0.01 M*
AgNPs + PPy	0.01 M*
Guava leaf extract	0.2 mg/mL**

** In which the concentration of AgNPs is calculated by the concentration of the reacted Ag^+ solution .** Calculated above (Figure 1)*

The procedure is as follows: Add 30 mL LB agar medium into each sterile petri dish; then add 100 µL of suspension for each type of bacteria (*E. coli* and *S. aureus*) and spread it evenly on the surface of the media plate. When the medium is solidified, use a 0.8 cm diameter agar perforator (d) to punch each of the 5-well plates and mark the wells (including control and material samples); Add 150 µl of each sample solution (solution containing the material created above) into the respective wells. The control well was filled with 150 µL of sterile distilled water (negative control) or isopropanol (positive control); Place the petri dishes in the refrigerator at 4^{o}C overnight so that the sample solutions diffuse evenly around the wells; Transfer the petri dishes to the incubator at 37^{o}C for 18 - 24 hours; Check the antibacterial activity of the sample solutions by measuring the diameter of the sterile ring around the well (D); Determination of the antibacterial activity of the sample solutions (D-d).

2.2. Results and discussion

2.2.1. Results of the synthesis of silver nanoparticles by guava leaf extract

Silver nanoparticles were fabricated by green synthesis as described in the methods section 2.1.2. To determine the formation of AgNPs, UV-Vis absorption spectroscopy and particle size distribution examination by DLS dynamic light scattering were applied.

Figure 1 shows the UV-Vis absorption spectrum of silver nanoparticles prepared by green synthesis from guava leaf extracts with an absorption spectral range between 300 and 600 nm, which is typical for the surface plasmon effect of the nanoparticles of Ag (Figure 3) [19]. On UV-Vis images, there is still strong absorption of the residual extract with an absorption peak of about 240 nm [22].

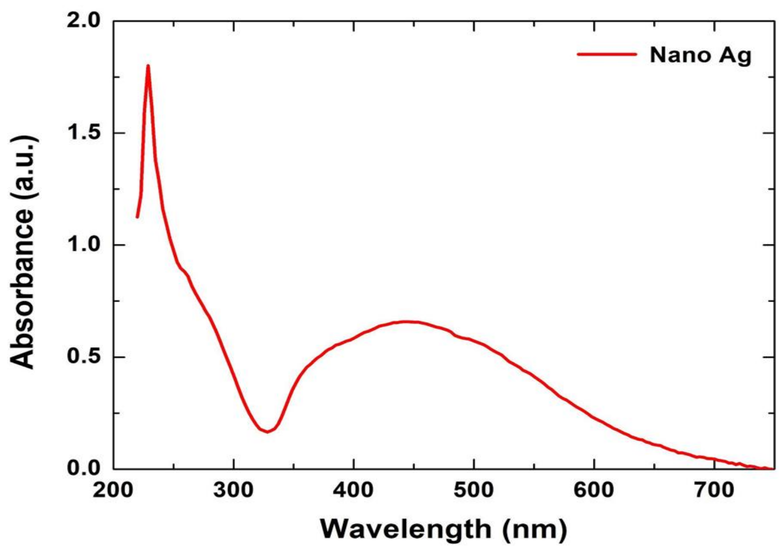

Figure 3. UV-Vis spectrum of AgNPs

Based on the UV-Vis spectrum of AgNPs materials synthesized by guava leaf extract, the diameter of the nanoparticles can be calculated according to the formula of Jain, S. and Mehata, M. S. (2017) as follows [23]:

$$d = \frac{hv_f}{\pi \Delta E_{1/2}} \quad (1)$$

Where h is Planck's constant, $v_f = 1.39 \times 10^6$ m/s is the Fermi velocity of electrons in bulk Ag, $\Delta E_{1/2}$ is the spectrum width at half-maximum (FWHM) in J units.

The results of the analysis of particle size by spectral width $\Delta E_{1/2}$ showed that silver AgNPs synthesized by guava leaf extract had a diameter of about 1.478 nm (d = 1.478 nm).

DLS measurement results showed that silver nanoparticles synthesized from guava extract had small and uniform sizes ranging from 1.1 nm to 1.5 nm (Figure 4). This result is consistent with the theoretical calculation results of silver nanoparticle size presented above.

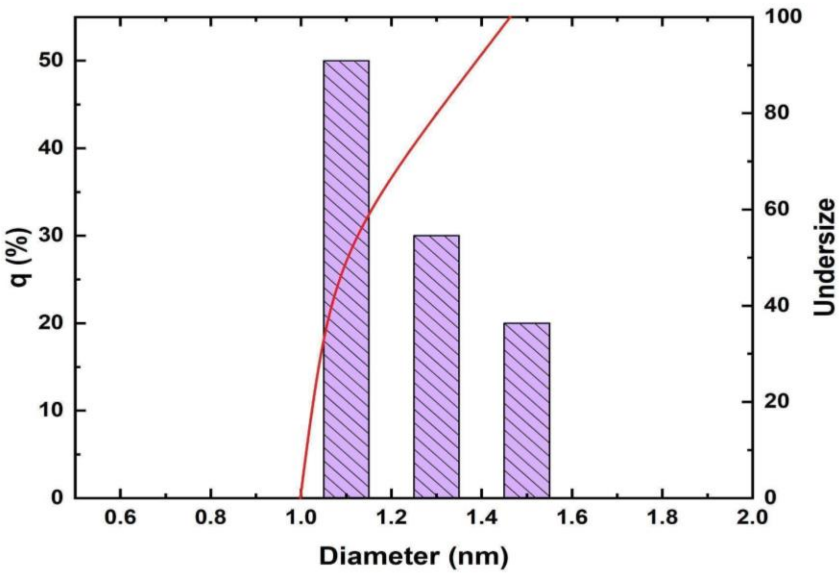

Figure 4. Particle size distribution of silver nanoparticles synthesized from guava leaf extract

2.2.2. Synthesis of water-soluble porphyrins (TASP)

The results of UV-Vis absorption spectrometry of water-soluble porphyrins (TASP) are shown in Figure 5. In this figure, TASP is characterized by a strong absorption peak in the Soret band (about 350 - 400 nm) and 3 absorption peaks in the Q-band (about 550 - 650 nm) (Figure 5).

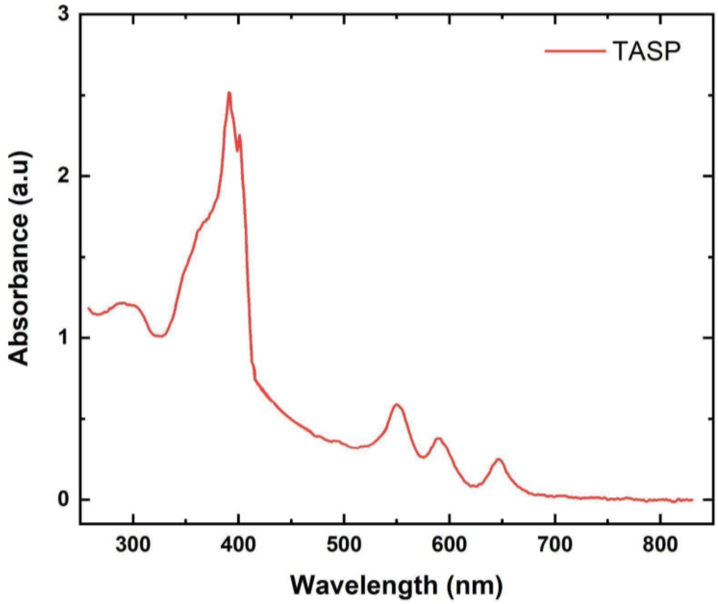

Figure 5. UV-Vis spectrum of water-soluble porphyrins (TASP)

2.2.3. Synthesis of AgNPs/TASP hybrid materials

The UV-Vis absorption spectrum of the AgNPs/TASP hybrid material sample shows the appearance of all characteristic absorption peaks of the component materials including the Soret and Q-band bands of TASP and the broad absorption band of silver nanoparticles from 300 to 600 nm (Figure 6).

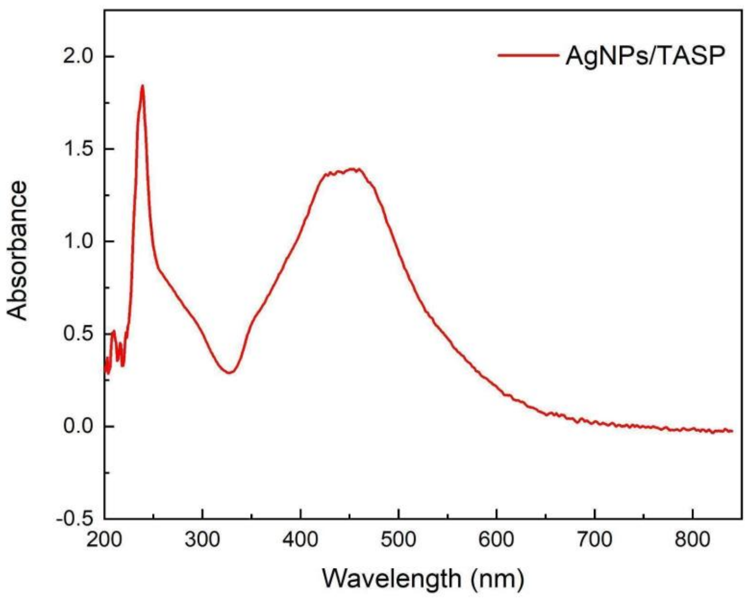

Figure 6. UV-Vis spectrum of AgNPs/TASP hybrid materials

Based on the UV-Vis spectrum of AgNPs/TASP hybrid materials, the diameter of the nanoparticles can be calculated according to formula (1) as above. The results of particle size analysis by spectral width $\Delta E_{1/2}$ showed that the AgNPs/TASP hybrid material prepared by green synthesis of AgNPs from guava leaf extract had a diameter of about 1.24 nm (d = 1.24 nm).

2.2.4. Antibacterial activity

The antibacterial ability of the created materials was evaluated based on the ability to inhibit the growth of *E. coli* and *S. aureus* through the data on the diameter of the sterile ring obtained in each test sample (Table 2, Figure 7). Figure 8 is a graphical representation of the results of the measurement of the sterile ring diameter of each test specimen.

Table 2. Antibacterial activity test results

Materials	**Anti-*E. coli*. Activity (D-d, cm)**	**Activity against *S. aureus* (D-d, cm)**
AgNPs	2.00	1.20
AgNPs/TASP	2.00	2.00
TASP	0.00	0.00
AgNPs/TASP+ PPy	2.90	1.45
AgNPs+PPy	2.70	1.05
Guava leaf extract	1.10	0.90
Negative control (sterilized distilled water)	0.00	0.00
Positive control (isopropanol)	1.90	1.70

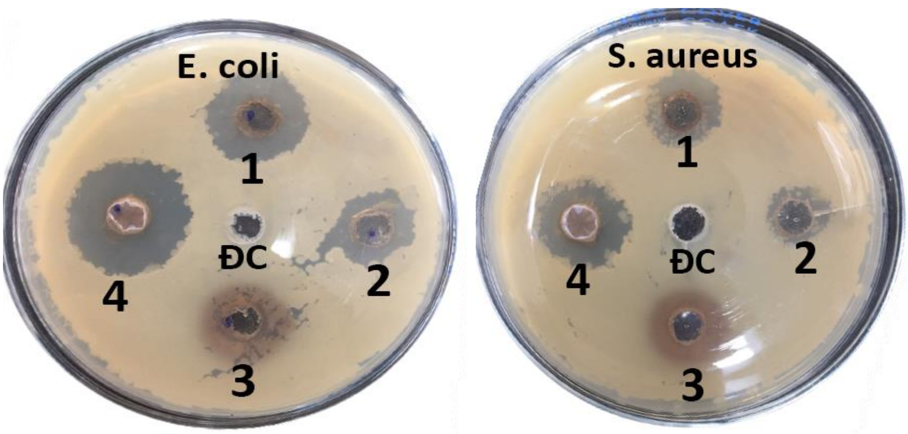

Figure 7. The image illustrates the test results for the antibacterial ability of S. aureus and E. coli of the materials. Control: Negative control; 1: AgNPs/TASP; 2: isopropanol; 3: AgNPs; 4: AgNPs/TASP + polypyrrole

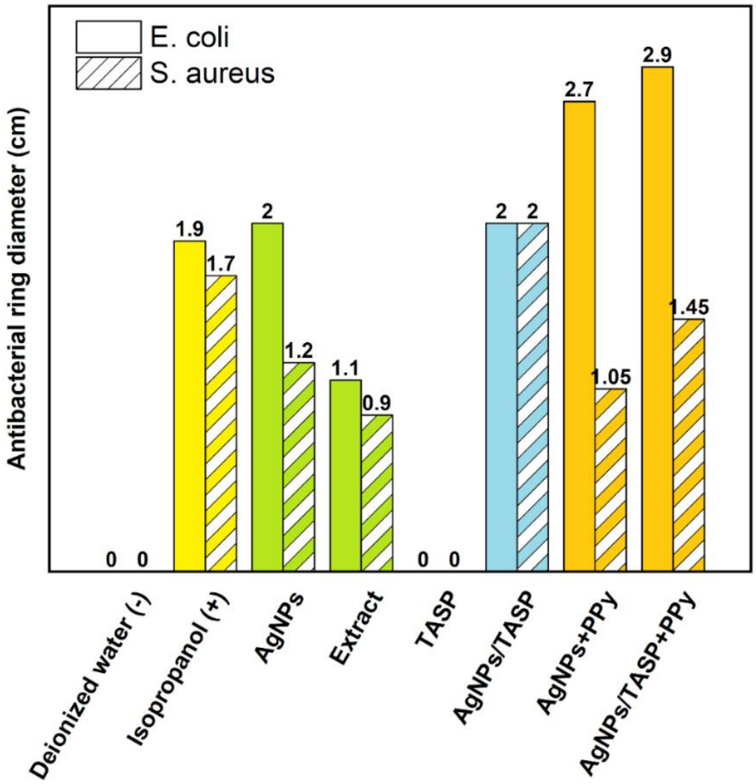

Figure 8. Graph showing antibacterial test results

The negative control (without antibacterial activity) and the positive control (with antibacterial activity) showed reliable test results of the samples.

AgNPs samples and guava leaf extracts both showed antibacterial activity. For the AgNPs test sample, the activity against E. coli (diameter of the sterile ring was 2.0 cm) was higher than that of *S. aureus* (diameter of the sterile ring was 1.2 cm).

According to studies, porphyrins have antibacterial activity only when activated by irradiation [13]. In this experiment, the water-soluble porphyrin sample TASP did not show antibacterial activity against either test strain. This is consistent with previously published research results. Meanwhile, the AgNPs/TASP hybrid material showed strong and the same activity against both tested bacterial strains (the diameter of the sterile ring was 2 cm). It can be seen that the hybrid material has better activity with a variety of bacteria than the conventional silver nanoparticle.

Both nanosilver samples and hybrid materials with the presence of polypyrrole, a polymer capable of promoting the kinetics of silver ion release showed strong antibacterial activity and were superior to nanosilver in species *E. coli* and *S. aureus*. In which, the hybrid material sample with the addition of polypyrrole (diameter of sterile ring 2.9 cm for *E. coli* and 1.45 cm for *S. aureus*) still gave stronger activity than the sample with only nano silver and polypyrrole (sterile ring diameter 2.7 cm for E. coli and 1.05 cm for S. aureus).

3. Conclusions

In this work, silver nanoparticles, water-soluble porphyrins (TASP), polypyrrole, and some hybrid materials (AgNPs/TASP, AgNPs+PPy, AgNPs/TASP+PPy) have been successfully synthesized and characterized by some techniques: UV-Vis and DLS methods. The UV-vis spectra of TASP show two characteristic absorption spectral peaks: Soret (350 - 400 nm) and Q-band (550 - 650 nm). The UV-Vis spectrum of silver nanoparticles has a broad absorption band of 300 - 600 nm, which is typical for the surface plasmon resonance (SPR) of silver nanoparticles (AgNPs). In the absorption spectrum of the hybrid material AgNPs/TASP Soret band, Q-bands, and plasmon resonance peak of pristine materials were overlapped.

The antibacterial activity of synthesized materials was tested on gram-negative and gram-positive bacterial strains. Hybrid materials between TASP (a water-soluble porphyrin derivative) and AgNPs are more resistant to *E. coli* and *S. aureus* than AgNPs. However, when adding polypyrrole (a polymer capable of promoting the release of Ag ions) to the AgNPs/TASP hybrid material, the antibacterial ability of this hybrid material was the highest among all the tested samples. This will open the direction of research, fabrication, and application of porphyrin-silver nanoparticle-polymers hybrid materials to increase antibacterial ability compared to conventional AgNPs [24].

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