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Enhancing the prodigiosin pigment by adding Ag\TiO_2 synergism for antibacterial activity

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Abstract:

To treat diseases brought on by drug-resistant microbes, researchers are presently using nanotechnology as one of the tools for studying the most obscure cornersof medical sciences. The biological synthesis regarding Ag/TiO2 synergism in prodigiosin pigment was the primary focus of the presented work. Inhibitory and bactericidal properties of silver nanoparticles (AgNPs) have long been recognized. Titanium dioxide nanoparticles (TiO2 NPs) are employed as antibacterial agents in a variety of therapeutic sources. With using Nd:Yag laser (1064nm), the composition has been synthesized by using environmentally friendly laser ablation in a liquid method. UV-visible spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) have been utilized for the analysis of the synthesized NPs. Optimal biosynthesis results showed a rise in the intensity of Surface Plasmon Resonance (SPR) bands of the NPs with shifting at 300 nm. TiO2 absorption peak at 230 nm. The results showed that the UV visible absorbance regarding prodigiosin pigment was at OD529. AgNPs/TiO2 exerted maximum antibacterial activity towards (*Streptococcus, Bacillus subtilis*, *E. coli,Proteus vulgaris*) Gram-negative and Gram-positive bacteria. The various Gram-negative and Gram-positive bacteria were subjected to various treatments (prodigiosin with Ag, prodigiosin pigment, and prodigiosin synergism with Ag/TiO2). The synergism is the best for antimicrobials than each one alone.

Keywords: antibacterial, nanoparticles, prodigiosin, and synergism.

Highlights

- Prodigiosin, a biological pigment, can be used to mediate the formation of silver nanoparticles.
- Nano compounds derived from the addition of prodigiosin pigment have been shown to improve antibacterial inhibition.
- TiO2 has a synergistic impact on the nanoparticle product made from silver particles and prodigiosin pigment.

Introduction

Over the last two centuries, antibiotic discovery has transformed the pharmaceutical sector, saving millions of lives (Uddin et al., 2021). The rapid and significant co-evolution of microorganisms against antibiotics has reduced the efficacy of these remarkable therapies,' generating concerns about drug resistance throughout the world. The World Health Organization (WHO) has admitted that the world is sort of out of drugs (Aljeldah, 2022). Efforts to find inventive antibiotics are undoubtedly slower than how microorganisms evolve and respond to antibiotics presently on the market. To deal with the repercussions, new processes or alternatives must be developed as quickly as feasible (Wozniak & Grinholc, 2018).

Most of the drug compound is produced from microbial metabolites or analogs of these substances (Abdel-Razek et al., 2020). On the other hand, standard medicine has lately been demonstrated to be ineffective against resistant pathogenic microorganisms. As a result, scientists and pharmaceutical firms are looking deeper into the origins of bacteria, particularly in the quest for novel bacterial candidates with unique characteristics in medications that may be effective against infections and drug manufacture (Murugaiyan et al., 2022).

Prodigiosins are oligopyrrol-pigmented antibiotics with substantial immunosuppressive and tumor-fighting activity (Bilek et al., 2020). Due to its antimalarial, anticancer, antibacterial, and antifungal properties, prodigiosin has long attracted study interest (Alaa & Hassan, 2021; Darshan & Manonmani, 2015; Lapenda et al., 2020; Yip et al., 2021). Both Gram-negative and Gram-positive bacteria, such as *Streptomyces* spp., and *Serratia* spp., can generate prodigiosin (Wang et al., 2020). The synthesis of anti-microbial red pigment is dependent on environmental factors and might be regulated by higher factors to achieve microbial ecology dominance over competing species (Hamza L A Yaaqoob Researcher Asist, n.d.; Karthika et al., 2015).

Inorganic particle administration in the synthesis of microbial metabolites has been shown to increase the antimicrobial ability of the particles produced (Dozie-Nwachukwu et al., 2017). Utilizing large-sized substances offers significant hurdles, including in vivo unsteadiness, restricted bioavailability and dissolution, insufficient absorption in the body, concerns with target-specific distribution and tonic efficacy, and potential adverse pharmacological effects. The resulting metabolite in nano size has the advantage to produce materials with new functions and better quality. Nano-sized metabolite products will boost spreadability, making it simpler to reach targets. The simplicity of reaching a target will be followed by a speedier reaction process, just like crushed ice cools a glass of boiling water more quickly than ice cubes. Because they are on an atomic scale, nanoparticles can more easily permeate membranes and interact with targets. The nanomaterial coating technique has shown to be an option for stabilizing materials to reach and react with objectives. Nano size can also reduce the number of doses administered, minimizing medication adverse effects on the host, particularly humans (Bano et al., 2023). Therefore, the addition of prodigiosin is suspected to increase NP activity. By far TiO_2 NPs are known to block the action of pigments. TiO2 NPs have historically been viewed as low toxicity, poorly soluble particles. They have traditionally been used as a "negative control" in numerous in vitro and in vivo particle toxicological studies (Karthika et al., 2015). Depending on how its crystals are arranged, titanium dioxide is either anatase or rutile. The small particle size, low light absorption, and high light reflection of this pigment make it perfect for creating high opacity. These pigments are extremely brilliantly white when they are finely separated. Due to this property, titanium dioxide pigments play a significant factor in the optical performance of paper's brightness and opacity, even at low concentration levels. Numerous studies have discussed the characteristics of TiO2 NPs and how to use them to reduce inorganic ions and degrade chemicals in aqueous solutions (Ibraheem et al., 2021; S. Jaber et al., 2021). However, the effect of TiO_2 on the synthesis of AgNPs mediated by the pigment prodigiosin is unknown. TiO_2 can be defined as a solid inorganic material which is a metal oxide with a white color that is less soluble, non-flammable, and thermally stable. Nanotechnology is now used in practically every industry, from textiles to electronics and medicines. The inhibitory and bactericidal properties of AgNPs have long been recognized (Ibraheem et al., 2021; S. Jaber et al., 2021). The antibacterial activity of AgNPs can be used in biomedical applications to treat various infectious disorders, prevent bacterial colonization of catheters, remove microbes from textiles, and disinfect water. The purpose of this study was to assess the characterization and antibacterial activity related to the synergism of TiO_2/AgNP produced by laser ablation in double distilled water to create a novel prodigiosin composition (Islan et al., 2022a).

Material and methods

Preparation of the prodigiosin pigment

Screening for pigment prodigiosin production by *Serratia marcescens* isolates

Between August and December 2019, 120 burn and wound swab specimens were collected and cultivated on MacConkey agar by laboratories at Al-Kindi hospital, Al-Yarmook hospital, and Baghdad Medical City Teaching Hospital. Isolates were acquired from these facilities by subculturing on MacConkey agar and then incubating at 37 degrees Celsius for 18-24 hours. Serratia marcescens isolates that had been validated by identification were tested for prodigiosin production in liquid culture in order to choose the isolate that generated the greatest prodigiosin.

Preparation of inoculums

Serratia marcescens isolates were generated as follows: 20 ml of the Brain heart broth and a few loops of the *S. marcescens* growth on nutrient agar from an overnight culture were added to a 150 ml Erlenmeyer flask. Such a culture was cultured for 24 hours at a temperature of 30 Celsius in an incubator(Arifiyanto et al., 2021).

Cultivation method

Amount of 50 ml of the minerals salt broth medium was prepared and then autoclaved at a temperature of 121 Celsius for 15 minutes in each of the 250 ml Erlenmeyer flasks. Each Serratia isolate has been inoculated with 1 ml (2% inoculum) of inoculum in each flask. After that, flasks have been incubated in an orbital shaker at a temperature of 30 Celsius and 200 rpm for a period of 48 hrs. Following incubation, samples were collected for prodigiosin analysis (Kaira et al., 2015).

Partial purification of prodigiosin pigment

250 ml of *S. marcescens* cell-free broth culture was collected following 30hr of the incubation, and the crude Prodigiosin has been aspirated from it. The culture medium has been first centrifuged for 15 minutes s at 8000 rpm. The collectedcells have been vigorously mixed with 250 ml of methanol for at least 3 hours at room temperature while the supernatant has been ignored. The mixture was after that centrifuged at 10,000 rpm for 20 minutes. A filter paper (0.2 µm, Whatman) was used to collect and filter the supernatant. The methanol filtrate was concentrated using a rotary evaporator at a temperature of 70 Celsius, and the red pigment was after that extracted using twice as much chloroform. In a separatory funnel, the two solvents have been vigorously mixed. The organic phase (chloroform phase) has been collected and dried at a temperature of 45 Celsius. The final pigment was ground into a powder, diluted in water using an ultrasonic device, and stored in a dark bottle in the refrigerator for an antimicrobial test. According to Chen and coworkers, Prodigiosin was purified partially (Chen et al., 2013a).

Prodigiosin pigment production

For comparative pigment production, liquid media were examined for the generation of pigments. A fresh 5% culture was briefly added to 20 ml of mineral salt broth. Prodigiosin production was assessed after 72 hours of incubation in the media. Through estimating the absorbance at 530 nm with the use of a double beam UV-Vis spectrophotometer as proposed by (Wei & Chen, 2005), the measurable determination from the red pigment was completed. 5 ml of additional broth and 4ml of methanol were used in the extraction process. For 2 mins, the mixture has been energetically vortexed. After that, the solution was centrifuged at 6000 rpm for 10 mins. Following the previous step, 0.80ml of supernatant has been mixed with 0.20ml of a 4:1 v/v mixture of 0.05N HCl and methanol.

Preparation of the NPs Samples

The Nd:Yag laser (740mJ, 100pulses) was used to generate the concentrate TiO_2 solution in the first step of the three-step pulse laser ablation in liquid process. The target was a 99.8% pure TiO_2 substrate with a 2 mm thickness. The silver nanoparticles (99%) with a thickness of 3 mm were employed to make AgNPs (740 mJ, 100 pulses) for prodigiosin. Then, silver nanoparticles (sliver NPs) were used to create Ag-TiO_2 synergism when submerged in concentrate colloidal TiO2. To avoid etching the substrate surface throughout the preparation procedure, the target was continually rotated (Islan et al., 2022b).

Nanoparticle Characterizations

A nanoparticle was characterized by following steps such as Scanning Electron Microscopy (SEM): (MIRA3 TSCAN) at 15 kV, SEM magnification 5 Kx with 200nm, X-ray Diffraction (XRD) Pattern: philps (PW 1730), Cu-Kα radiation source at 2 θ angles = (10–76) degree, UV-visible spectrum:(8,000 series, Shimadzu) supplied from Japan has been utilized in a spectral range of 400-4000cm^{-1}. All measurements were done at the sniper lab, Baghdad- Adhamiyah near al- Nu'man Hospital, and Collection bacteria isolates: The specimens have been collected by the labs at Al- Kindi Hospital, Teaching Hospital of Baghdad Medical City, and Al-Yarmook Hospital.

Identification of bacteria isolates by Vitek -2 system

The bacterial isolates were identified using Vitek-2- sensitivity test. It is a two- parts compacted system (computer and instrument). It is an entire system for the routine identification of the most important non-fermenting and fermenting positive bacteria and Gram-negative. The preparation of solutions, incubation of samples before application, adjustment of the optical system, card sealing and incubation, application of test reactions, and analytical methods were all done following the company's established manufacturing procedures (Ling et al., 2003).

Result and Discussion

XRD Peak

The XRD peaks of silver NPs composition displays three peaks in a spectrum that ranges within (20 - 70). The XRD peak patterns were centered at (32°, 46°, 57° and 72°) at (111), (200), (220), (3 1 1) respectively, (JCPDS NO. 01-080-0075) (Lazic et al., 2022). The particles size estimation with the use of Scherer equation, $D = \frac{0.98\lambda}{\beta \mathrm{Cos}\theta}$ (1), in which D represent particle size, λ represent wavelength of Cu Kα source, β represent FWHM of the sliver NPs at diffraction angle (Gojznikar et al., 2022; Lazic et al., 2022)

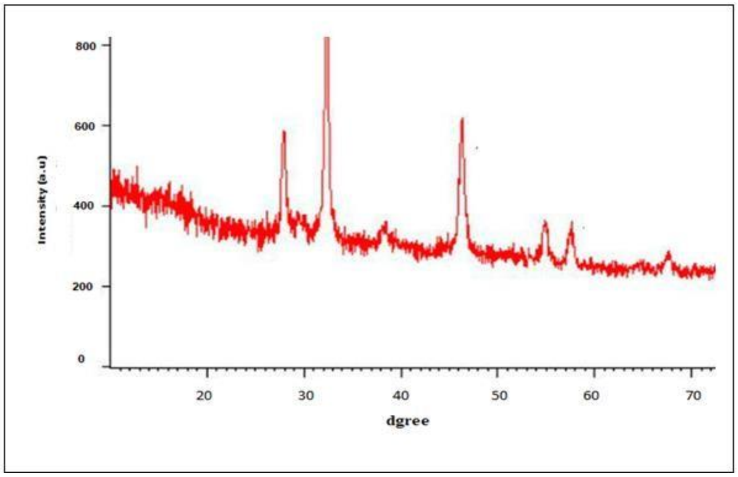

Figure 1. Diffraction peaks for AgNPs at (740mJ and 100 laser pulses).

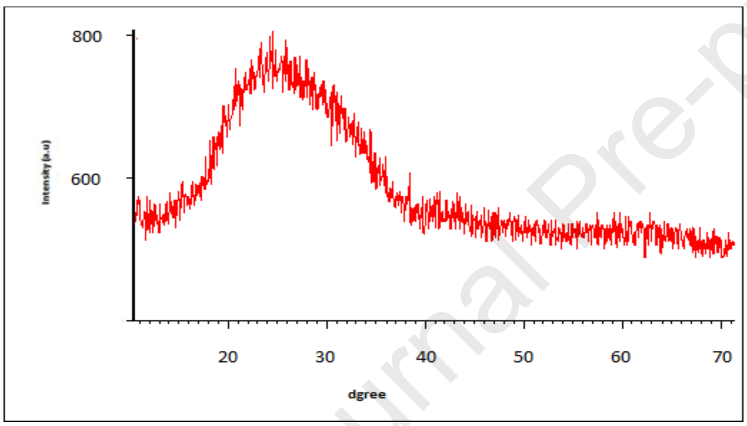

Figure 2. XRD-pattern for TiO_2 NPs at (740mJ and 100 laser pulses).

Laser synthesis produced the XRD pattern of TiO2 NPs that have seen shown in Fig. (2). All TiO2 NPs are in the anatase phase, according to XRD patterns. The card's JCPDS number, 21-1272, is in good accord. Peaks have been absorbed at 25^{o}, 38^{o}, 48^{o}, 53^{o}, 55^{o}, 62^{o}, and 75^{o}, with corresponding miller indices values of (1 0 1), (0 0 4), (2 0 0), (1 0 5), (2 1 1), (2 0 4) and (2 1 5), respectively. Comparable to nanomaterials, whichachieved lattice parameters a = b=0.3785nm & c=0.9513nm, particle size reduces as the peak width increases. The Debye-Schereer equation was used to get the average crystallite size, while the crystal size (25nm) for AgNPs (Khajavi Sh et al., n.d.) (Jaber et al., 2021).

UV-Visible Spectrum

One of the most important methods for identifying metal NPs is UV-Vis spectroscopy. SPR that results from coherent electron oscillations in the conduction band regarding NPs induced through electromagnetic field, is what causes absorption behavior. Due to the conduction electrons' collective oscillations, SPR develops when NPs are exposed to visible light (Anik et al., 2022). In our findings, a sharp peak at 300 nm was acquired through a UV-visible spectroscope specifically for the synthesis of AgNPs, as the Figure 3 (A). Colloidal AgNPs are widely known to exhibit absorption at a wavelength range of 300 to 420 nm because of Mie scattering (Shen et al., 2020). The absorption spectrum concerning TiO2NPs in DDW is illustrated in Figure 3(B), which also includes the absorption spectra at (230) nm (Lazic et al., 2022) . A UV- wavelength scan has been used to assess the absorbance value regarding prodigiosin, which was found to be at OD529 for UV visible pigment. Progiosin pigment has distinctive absorbance at 530nm (Gojznikar et al., 2022). Each sample extract stayed dissolved in the methanol, and the extract of the methanol had maximal absorbance at 538nm. The maximal spectrum of absorption of the prodigiosin was enhanced from Serratia spp. KH-95 was 535 nm according to (Song et al., 2006a), as shown in Figure (4).

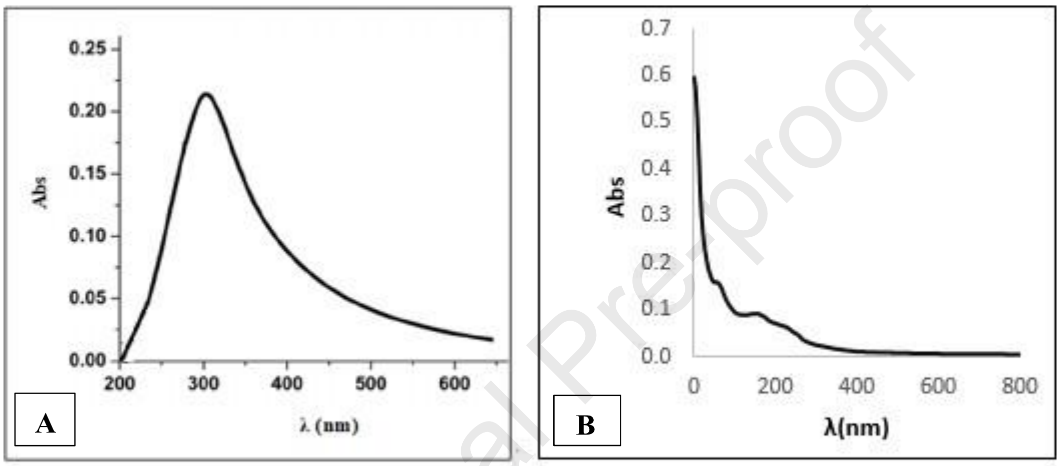

Figure 3. UV–VIS Spectral Analysis of (A) AgNPs in double distilled water and (B) TiO_2NPs in double distilled water Synthesized by laser ablation in liquid.

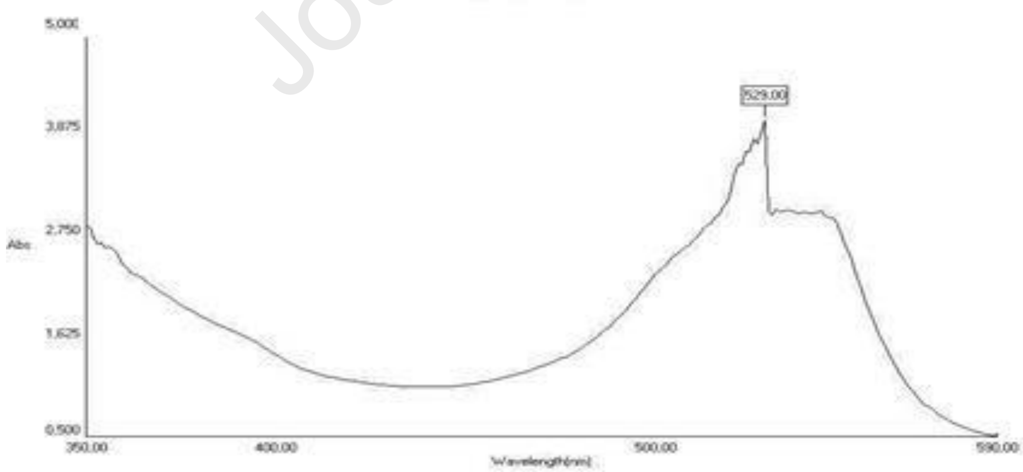

Figure 4. Prodigiosin pigment in UV-Visible about OD 530 nm.

SEM Image

Figure 5 shows SEM images of TiO2NPs, AgNPOs, and synergism Ag/TiO_2NPs prepared by laser at 740 mJ and 100 pulses in Prodigiosin pigment. The NPs were nearlyspherical in shape and homogeneous. Due to fixing laser mixing with pigment, a few areas of SEM images show NP agglomerates. The concentration of such tends to rise, as seen in (A) AgNPs, (B) TiO2NPs, and (C) the synergism distribution inside the pigment homogeneous. The production of the NPs and their morphological dimensions in the SEM research showed that the average size has been 50 nm with antiparticle distance, and the forms have been uniformed spherical and ellipsoidal (Mohajjel Shoja et al., 2021).

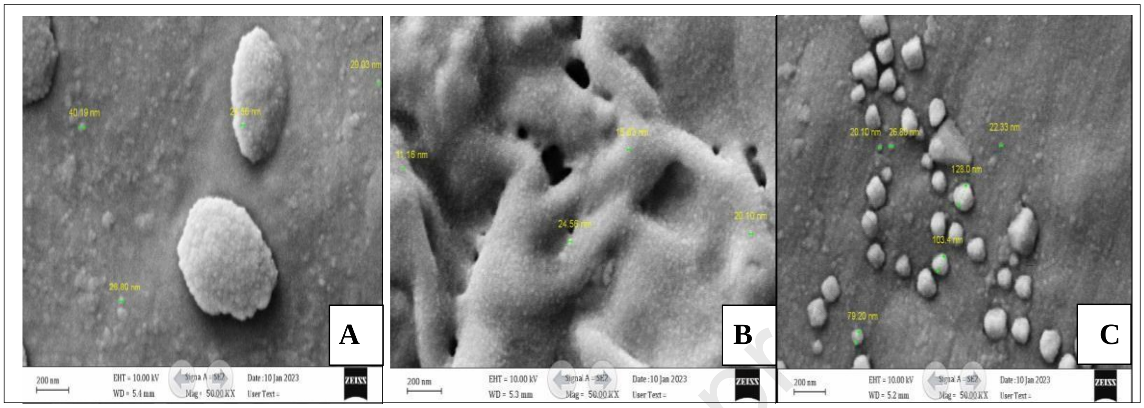

Figure 5. SEM image with 200nm magnification for (A) AgNP, (B)TiO2NP,s, and (C) Ag/TiO2 NPs synergism in Prodigiosin pigment.

Antibacterial Activity

In this study, four Gram-positive and negative bacterial isolates were used: *Escherichia coli*, *Streptococcus* sp., *Bacillus subtilis*, and *Proteus vulgaris*. The nanoparticle synthesis treatments were divided into three groups: prodigiosin pigment only, prodigiosin-AgNPs, and prodigiosin-Ag-TiO2-NPs. The results showed that the simultaneous use of prodigiosin-Ag-TiO2-NPs provided greater inhibition against all types of bacteria, with susceptibility for the greatest inhibitor of *B. subtilis* (Table 1).

The dimension, form, charges on the surface, quantity, and dispersion state are the most important physicochemical characteristics influencing AgNPs' antibacterial potential. AgNPs demonstrate antibacterial activity via a variety of methods. The most notable routes of antimicrobial activity of AgNPs have been identified as adherence to bacterial cells, penetration into the cells, ROS and free radical production, and regulation of microbial signaling pathways (Dakal et al., 2016). Nanoparticles that adhere to the surface of cells will disrupt microorganisms' cellular transport routes, stressing them with elevated metal levels, activating oxidative reactions, and leading to cell death.

These fundamental findings are compatible with studies demonstrating that the combination therapies reported empirically concluded that synergistic validity has been likely a key for avoiding antimicrobial resistance (Ni et al., 2013), then balancing those elements throughout the combination therapy (Chen et al., 2013b; Song et al., 2006b). The use of nano-sized materials and cellular products will facilitate transport across the membrane to imitate the capacity of cells to link up and communicate with physiological surroundings (Jiménez-Jiménez et al., 2020). As a consequence, the utilization of products derived from nanoparticle production in this study has the potential to be expanded further, particularly in terms of overcoming microbial resistance to antibiotics.

Research using extracts from bacterial metabolites to be used as an effort to produce antimicrobial products has developed rapidly. The use of extracts from bacteria is considered promising because of the short life cycle of bacteria and the low cost of production to obtain them (Arifiyanto et al., 2021). Other experiments using plant materials and nanoparticle manufacturing reveal that the trend of employing living things and their metabolites is highly promising (Asif et al., 2022). Unfortunately, if the medicinal plants utilized and their nutritional value are significant enough, it will be catastrophic if the prices are high and increased demand becomes a distinct barrier to their sustainability.

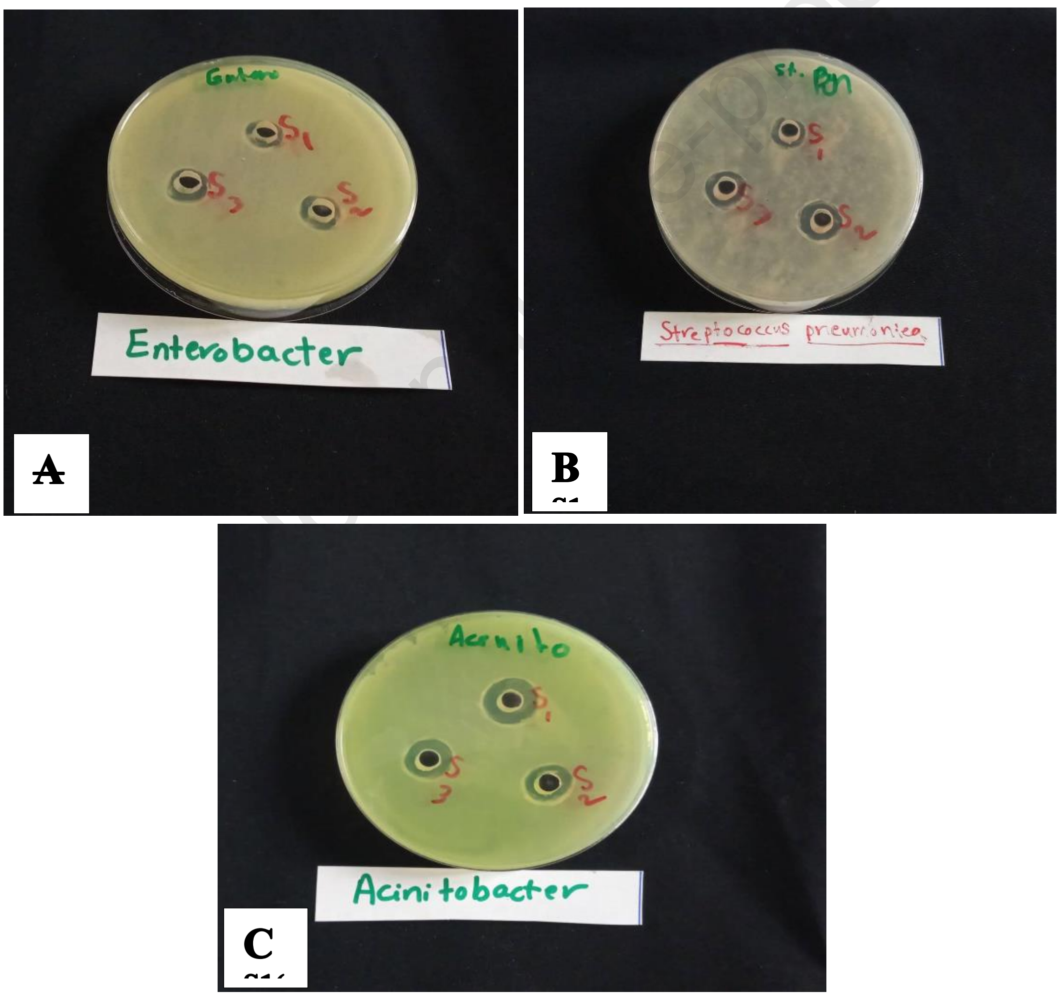

Figure 6. Antibacterial activity for (A)AgNPs, (B)TiO2NPs, and (C) Ag/TiO2NPs synergism in prodigiosin pigment against positive and negative bacteria Gram.

Table 1. Inhibition zone measurement to compare the result before and afteradding the NPs to the pigment.

Antibacterial	S1	S2	S3	S4
Prodigiosin	8	8	8	12
Prodigiosin synergism with Ag	9	9	9	13
Prodigiosin synergism with Ag/TiO_2	11	10	11	17

* S1-S4 are measured in millimeters

Statically Analysis

The chi-square test (χ^2) was used, which is a non-parametric test that measures the quality of reconciliation, independence, and homogeneity of the data, and by using the statistical program (SPSS 26). The value of the test was (5.388) significant in the level (0.041) which is less than (0.05). The effect of the species and also the difference of the type with the effect of bacteria in addition to the homogeneity and stability of the data and the quality of their reconciliation, tables (2 & 3).

Table 2. Descriptive Statistics

Antibacterial * Types of Crosstabulation Count		S1	S2	S3	S4	Total
Antibacterial	Prodigiosin	8	8	8	12	36
	Prodigiosin synergism with Ag	9	9	9	13	40
	Prodigiosin synergism with Ag/TiO2	11	10	11	17	49
Total		28	27	28	42	125

Table 3. Chi-Square Test

Chi-Square Tests			
Pearson Chi-Square	5.388[a]	6	0.041
Likelihood Ratio	5.688	6	0.041
Linear-by-Linear Association	4.014	1	0.044
N of Valid Cases	125		

a. 0 cells (0.0%) have an expected count of less than 5.

Conclusion

In summary, the enchantments of absorption and bacterial efficiency of prodigiosin pigment which has been used as antioxidant, antiviral, antibacterial, and anticancer activities by adding AgNPs and synergism Ag/TiO2 NPs prepared by laser ablation. The study showed the superiority of the synergism Ag/TiO2 NPs due high surface area and more antibacterial activity.

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Code availability

Not applicable.

CRediT author statement

GSJ: Conceptualization, Methodology, Validation, Data Curation, Project. **SSD:** Methodology, Formal analysis, Writing - Original Draft, Visualization. **TAAH:** Formal analysis, Data Curation, Writing - Review & Editing, Visualization, Supervision. **NAI:** Conceptualization, Supervision, Project administration, Funding acquisition. **AA:** Writing - Review & Editing, Supervision.

Declaration of competing interest

The authors state that they have no known conflicting financial or personal interests that may have seemed to affect the work presented in this study.

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Declaration of competing interest

The authors state that they have no known conflicting financial or personal interests that may have seemed to affect the work presented in this study.