Antimicrobial Activity of Biosynthesized Silver Nanoparticles using *Stenochlaena palustris* and its Synergistic Effect against Methicillin-Resistant *Staphylococcus aureus*

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Abstract

In this study, the combination of biosynthesized silver nanoparticles and clindamycin, a ribosomal antibiotic, was investigated by Kirby Bauer Disk Diffusion Assay. First, an aqueous extract of Hagnaya Fern was prepared to synthesize SNP from a silver nitrate solution. The nano-Ags were then subjected to UV-Vis spectroscopy. By the maximum absorbance at 400 nm, it was concluded that the biosynthesized SNPs are spherical in nature. The mean inhibition halo for biosynthesized SNP, clindamycin, and CSNP was 9.75 mm, 31.75 mm, and 36 mm, respectively. The results showed that silver nanoparticles derived using Stenochlaena palustris leaf extract are an effective adjuvant for clindamycin against MRSA and can be used for its synergism with the antibiotic.

Keywords: Silver nanoparticle; Biosynthesis; Stenochlaena palustris; MRSA

1. Introduction

The susceptibility of different subjects in the field of healthcare can be blamed for the prevalence of one of the emerging pathogens for nosocomial infection– the methicillin-resistant *Staphylococcus aureus.* For healthcare workers, daily exposure to the pathogen and any suspecting and possible samples introduces some risks. In addition, 54.34% of MRSA incidents are found in sputum samples– which puts laboratory personnel at a higher risk. On the other hand, open wounds, catheters, and the urinary tract are the potential infection sites for admitted patients (Mahmood, et.al., 2010).

The future of medicine against antibiotic-resistant pathogens is currently resting on the field of nanotechnology. It is a field that is being focused on today amid the antibiotic resistance crisis. It has gained appreciation from the scientific community because of its potential to be an antimicrobial agent. Silver nanoparticles are an important compound because of their ability to cause toxicity to microorganisms but prevent any biological alterations to human blood at certain low concentrations (Krajewski, et.al., 2013).

The green synthesis of silver nanoparticles is a method capable of replacing any previous physical or chemical synthesis. It is cheap, reliable, and answers the concern of the environment. This advancement uses biological materials as a reducing and stabilizing agent for the NPs (Ghotekar, et.al., 2020).

In this study, the researchers investigated the synergistic properties of biosynthesized silver nanoparticles derived using *Stenochlaena palustris* leaf extract. More specifically, its antimicrobial activity against Methicillin-resistant *Staphylococcus aureus.*

2. Methods

2.1. The subject of the study

Methicillin-resistant *Staphylococcus aureus* is a nosocomial pathogen that causes skin infections. It causes pneumonia, which is a serious morbidity and mortality rate in many hospitals worldwide (Boucher, et al., 2010). The purchase of

Stenochlaena palustris (Hagnaya) was made in Brgy. Danao McArthur, Leyte, Philippines. The Methicillin-resistant *Staphylococcus aureus* (MRSA) culture was tested against SNP. The experimentation was done at the PHINMA-Saint Jude College, Sampaloc, Manila City.

2.2 Leaf sample preparation

The collected leaves of *Stenochlaena palustris* were washed with distilled water to remove contaminants such as dust and debris. After that, the samples were dried out until it appeared brown.

2.3 Extraction

Dried leaves of *Stenochlaena palustris* were boiled in 100 ml of distilled water for 10 minutes. After that, the initial aqueous solution was filtered and stored until further use.

2.4 Biosynthesis of Silver nanoparticles

To synthesize our silver nanoparticles, the researchers produced 1mM of silver ions by dissolving 0.017 grams of silver nitrate in 100ml of distilled water. 10 ml of the prepared silver ions was mixed with 5 ml of *Stenochlaena palustris* leaf extract. The prepared solution was incubated for 1 hour, away from direct sunlight.

2.5 Silver nanoparticles characterization

To confirm the development of biosynthesized silver nanoparticles in the prepared extract-silver nitrate solution, the absorbance of the sample was read after incubation using UV-vis spectroscopy. A color change was observed to indicate the presence of SNP after incubation

2.6 Filtration and purification of silver nanoparticles

To prepare our 100% silver nanoparticles, the incubated solution was centrifuged at 10, 000 rpm for 30 minutes. After that, the formed pellets were resuspended in 1 ml of distilled water. This process was repeated 5 times to ensure the purity of the particles.

2.7 Antimicrobial activity of silver nanoparticles

Before the preparation of Petri dishes, blank paper discs and antibiotic-supplemented discs were impregnated with 20 µl of our silver nanoparticles solution (Meroni et al., 2020). The prepared discs stood for 5 minutes to fully absorb the solution. The antimicrobial activity of the solution was tested against Methicillin-resistant *Staphylococcus aureus.* First, a 0.5 McFarland standard suspension of MRSA was prepared. Then, a swab was dipped and the bacteria were cultured in Mueller-Hinton agar. After the streaks were allowed to dry, the plates were divided into four quadrants for the different antibiotic discs the researchers wished to use in this study: Silver nanoparticles, Clindamycin, Clindamycin + SNP, and distilled water The plates were incubated for 16-18 hrs, at 35C in accordance with the CLSI standard for MRSA.

3. Results and Discussion

3.1 UV-Visible spectroscopy

The silver nanoparticle-leaf extract solution was subjected to UV-visible spectroscopy (Table 1). Initially, the solution's visual characteristics changed after adding the leaf extract. The lowest absorbance was measured on the wavelength of 700 nm and the maximum absorbance was measured at 400 nm. Several studies have suggested that silver nanoparticles whose highest absorbance is at 400 nm are in a spherical shape (Jyoti. et al, 2015).

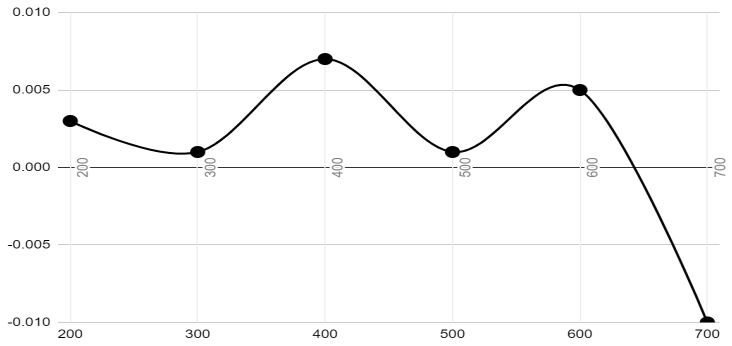

Fig. 1. Absorbance of SNP produced using the leaf extract of *Stenochlaena palustris* on different wavelengths

3.2 Antimicrobial Susceptibility Testing

Table 1. Kirby-Bauer Disk Diffusion Assay

	Zone of Inhibition (mm)							
Silver Nanoparticles	10	11	10	9	11	8	10	9
Clindamycin	31	34	30	30	31	33	32	33
Combination	37	35	38	34	35	35	36	38

Statistical differences were found in the mean inhibition halo of the silver nanoparticles, clindamycin, and the combination of both (9.75 mm, 31.75 mm, 36 mm, respectively). The combination of the biosynthesized silver nanoparticles and clindamycin showed the greatest inhibition compared to the result of the silver nanoparticles and clindamycin alone. The significant test result was found by performing a One-Way Analysis of Variance ($p \leq 0.05$). (Table 2)

Table 2. One-way ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3176.333333	2	1588.167	855.1666667	7.59E-21	3.466800112
Within Groups	39	21	1.857143			
Total	3215.333333	23				

After finding out that there is a significant difference between groups, a post-hoc test by the Bonferroni method was performed to come up with data that would determine if there is a significant statistical difference between each pair of groups ($p \leq 0.0167$) (Table 3). The computed P-values between the three groups were all extremely lower than the corrected Bonferroni P-value of 0.0167 which would deem the differences significant.

Table 3. Post-Hoc Test (Bonferroni Method)

Groups	P-value	Significant?
SNP vs Clindamycin	6.47476E-15	Yes
Clindamycin vs Combination	5.81781E-05	Yes
SNP vs Combination	6.48895E-16	Yes

4. Conclusion

There is a significant difference between the zone of inhibition between the combined clindamycin and silver nanoparticles than when it is used alone because of the computed P-value (5.818×10^{-5}) that is less than 0.0167– which signifies a remarkable synergistic effect between the two. The biosynthesized silver nanoparticles act as an adjuvant to clindamycin by enhancing the production of hydroxy radicals– which is a mechanism unusual for ribosomal antibiotics. (Hwang et al., 2012)

Acknowledgement

The researchers would like to thank the people who supervised the research and to the facility of Saint Jude College, Manila that made this study possible.

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