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Antimicrobial property of bio-reduced silver nanoparticle prepared using aqueous fruit extract of Antidesma bunius L. Spreng against Methicillin-resistant and susceptible Staphylococcus aureus

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Abstract – *With the rampant spread of highly drug-resistant bacterial pathogens along with the poor research and development for new antimicrobials and prospects for new antimicrobial agents, a call for antibiotic alternatives are now in demand. Antidesma bunius is being recognized due to its bioactive components which exhibits notable pharmacologic properties. Among different fruits, certain studies revealed that A. bunius have antibacterial properties, α-glucosidase inhibitory activities, anti-diabetic properties, and antioxidant properties. This study determined the antimicrobial activity of silver nanoparticle (Ag NP) bio-reduced by A. bunius fruit extract against methicillin-resistant and susceptible Staphylococcus aureus (MRSA and MSSA). Biosynthesized nanoparticles were characterized, and their antimicrobial properties were determined using disc diffusion and tube dilution assay. Results show that the synthesized Ag NPs are active against MSSA and the five clinical isolates of MRSA indicating potential antimicrobial activity. The MIC of MSSA was 0.38 to 1.52mg/mL and 0.38 to 0.76 mg/mL for MRSA. The MBC of MRSA and MSSA were 0.38 mg/mL and 0.76mg/mL, respectively. The synthesized silver nanoparticles can inhibit MSSA and MRSA, however, their inhibitory activity is lesser than that produced by the standard antibiotics. This study provides a potential, natural antimicrobial agent against inhibiting Staphylococcus aureus infections.*

Keywords –*Antidesma bunius, multidrug-resistant organisms, MIC, MBC, MSSA, MRSA, nanoparticles*

INTRODUCTION

With the growing threat of new antimicrobial resistance mechanisms emerging and spreading globally, millions of lives are lost each year due to infections and diseases caused by bacteria which could not be eliminated. Most people fail to complete a full course of prescribed antibiotics; thus, giving the bacteria a chance to survive as well as to adapt to an environment with low concentrations of antibiotics. There are a number of actively pursued strategies, including searching for new antimicrobials from natural products, modification of existing antibiotic classes, and the development of antimicrobial peptides [1]. Through the revolutionary improvement of microscopes in the past decades, the search for the tiniest particle became possible.

In recent years, there is an introduction of a new science called nanotechnology. This innovation is a science, technology and engineering which deals with the study of structures between 1 nanometer and 100 nanometers in size. According to Sadowski [2], particles which cannot be seen by the naked eye alone were made possible to be manipulated and redesigned in terms of its structure and use through the introduction of nanotechnology. This also opened a lot of possibilities in the research and development of different areas and fields of science [3]. One area that will surely benefit from nanotechnology is the field of medicine. In recent years, metal nanoparticles had been increasingly used in many sectors of the economy, such as the research for how nanotechnology can help in possibly treating diseases [4]. However, there is growing interest in the biological and environmental safety of their production. The main methods for nanoparticle production are chemical and physical approaches that are often costly and potentially harmful to the environment [5]. Thus, the use of plant extracts to

synthesize metal nanoparticles had been practiced nowadays.

Silver (Ag) is a soft, white, lustrous transition metal. It exhibits the highest electrical conductivity, thermal conductivity, and reflectivity of any metal. Silver nanoparticles are widely used in medical field including diagnosis, treatment, drug delivery, medical device coating, and for personal health care. It has been proven and recognized as having an inhibitory effect towards microbes commonly present in medical and industrial processes [6].

Antidesma bunius L. Spreng or bignay, a member of family Phyllanthaceae, is a fruit tree that grows best in the hot, humid tropical lowlands. It is native in the Philippines which normally flowers during the rainy season and bears fruits in the late rainy season that is during August to September. It is mainly found in rain forests and semi-evergreen seasonal forests. It is found in open places and secondary forests and grows in the wild [7]. A. bunius has a variety of uses. Its leaves have been traditionally used in treating snake bites. The bark produces strong fiber for rope and cordage. Its round-shaped fruits in a cluster of 30-45 per bundle turn from color green to red to black as it becomes ripe [8]. The fruit has a sour taste when unripe and sweet when ripe. The potential of the extracts of this fruit as an antibiotic alternative is gaining recognition due to its high phenolic property which exhibits antibacterial properties [9], α-glucosidase inhibitory activities [10], antidiabetic properties [11] and notable antioxidant properties [8]. Staphylococcus aureus is a gram-positive bacterium that is becoming resistant to antibiotics.

According to Hawkey [12], with the first usage and introduction of antimicrobials, the burden of resistance among bacteria has progressively increased within the last 10 years due to their misuse and overuse, one of which is penicillin. Penicillinase production happened rapidly globally. Then followed by the accumulation of resistance to tetracycline, vancomycin and macrolides and creates strains which caused problems in the treatment and management of nosocomial infections. But it was resolved through the introduction of methicillin before the marked increase of methicillin- resistant S. aureus along with methicillin- susceptible S. aureus came to be. MRSA resists medicines that usually treat infections. According to Center for Disease Control and Prevention, MSSA infections can cause toxic shock syndrome, cellulitis, staph food poisoning, folliculitis, boils, impetigo, and scalded skin syndrome. Up to date, no study has been reported about the antimicrobial property of silver nanoparticle using fruit extract of A. bunius against MRSA and MSSA.

Alongside with the fast-paced advancement of today's medical technology, the usage of metallic nanoparticles as an alternative has been progressing over the recent years, labelling Nanomedicine as promising and something to look forward to hereafter.

Antibiotics are effective in treating bacterial contamination. However, extensive use of these substances may lead in increasingly antibiotic-resistant bacteria. The aim of this study is to identify if silver nanoparticle can be reduced by Antidesma bunius L. Spreng and determine if the synthesized nanoparticle can effectively exhibit antimicrobial property against MSSA and MRSA; thus, helping in the development of antibiotic alternatives which are not hazardous nor overall difficult to produce.

MATERIALS AND METHODS

Plant material

Figure 1. Unripe *A. bunius* fruit

A. bunius L. Spreng fruit (Figure 1) was collected from Sto. Tomas, Batangas. Voucher samples of the plant specimen were sent to the Bureau of Plant Industry, Malate, Manila for authentication, The unripe fruits were chosen and used in this study.

Aqueous fruit extract preparation

Fifty grams of unripe A. bunius fruit were weighed and washed thoroughly with distilled water. They were crushed and a 200 mL sterile distilled water was added then blended. The mixture was filtered into a muslin cloth three times until it has a watery consistency. This was then filtered twice using Whatman No.1 paper as shown in Figure 2 [13], [14].

Figure 2. Filtration of *A. bunius* extract.

Figure 3. Five isolates of Methicillin- resistant *S. aureus* (MRSA)

Figure 4. Methicillin-susceptible *S. aureus* (MSSA)

Test strains

The organisms used were five isolates of Methicillin-resistant S. aureus (Figure 3) which was obtained and cultured at Batangas Medical Center and were confirmed at Mary Mediatrix Medical Center, Lipa Batangas. Similarly, Methicillin-susceptible S. aureus ATCC 29213 (Figure 4) was obtained at Batangas Medical Center.

Synthesis of silver nanoparticles

A 10 mL of A. bunius fruit extract was added into 90 mL of aqueous solution of 0.01M silver nitrate (Figure 5) for reduction into silver ion. It was exposed to bright sunlight within an hour until the change in color from yellowish to reddish brown color is achieved [15].

Figure 5. 0.01M silver nitrate solution and reddish-brown colored product.

UV-Visible (UV-VIS) Spectroscopy

The maximum absorbance of Ag NPs was tested using an ultraviolet-visible spectrophotometer UH5300 Hitachi. This instrument was used to analyze and determine the optical property of the synthesized nanoparticle [16].

Computations

The concentration of silver nanoparticles in the tube dilution was calculated using molarity which is the concentration of a particular chemical species in a solution (Harvey, 2000):

$$\text{Molarity} = \frac{\text{Number of moles}}{\text{Volume in liters}}$$

Thus, using the formula, the desired equation was derived as follows:

Moles = Molarity x Volume in liters

Afterwards, the number of moles was multiplied with the molar mass of the metal alloys used:

Mass of metal alloy in grams = Moles x molar mass of metal (g) mol

The resulting value in grams was converted to micrograms (µg). The value acquired was then divided with the volume of unripe *A. bunius* (bignay) fruit extract used in the synthetization of the nanoparticles to obtain the concentration of metal alloy present in the solution for each dilution:

$$\text{Concentration in µg/mL} = \frac{\text{Mass of metal alloy in ug}}{\text{Volume of bignay extract in mL}}$$

Disc diffusion method

The antibacterial activity of Ag NPs against MSSA and MRSA was carried out using Kirby-Bauer disk diffusion susceptibility test method. Using sterile cotton swabs, the bacteria strains were spread at the Mueller-Hinton agar. Afterwards, antibiotic disks including oxacillin, vancomycin, and cefoxitin were impregnated, serving as positive controls. Sterile blank disks were then loaded with 20 µL of Ag NPs solution. Distilled water-loaded disks served as vehicle control. The disks were then placed on the agar plates which were then incubated at 37ºC for 24 hours. The zone of inhibition was read and measured (in mm) and was then taken as the antibacterial efficacy of the nanoparticles against the tested organisms [17].

Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) determination

A macrobroth dilution as shown in Figure 6 was employed to determine the MIC of Ag NP against MRSA and MSSA. Thirteen (13) sterilized tubes were used in this procedure. A 1.0 mL of Mueller-Hinton broth was transferred into Tubes 2 to 11. To Tube 12, 2.0 mL of Mueller-Hinton broth were added. Tubes 1 and 2 were added with 1.0 mL of Ag NPs then serial dilution was performed. A 1.0 mL of the 0.9% NSS with 10ul loop of MRSA or MSSA which was compared first to McFarland Solution to standardize the application of the bacteria and was added into Tubes 1 to 11 and 13, respectively. To Tube 13, 1.0 mL of the antibiotic standard (Vancomycin) was added. The tubes were incubated at 37ºC for 24 hours. After the incubation period, the tubes were examined for growth of the microorganism. The indication to determine if there was a growth was turbidity of the tubes. The tube with the lowest concentration of metal nanoparticle at which no growth was observed was reported as the MIC [18].

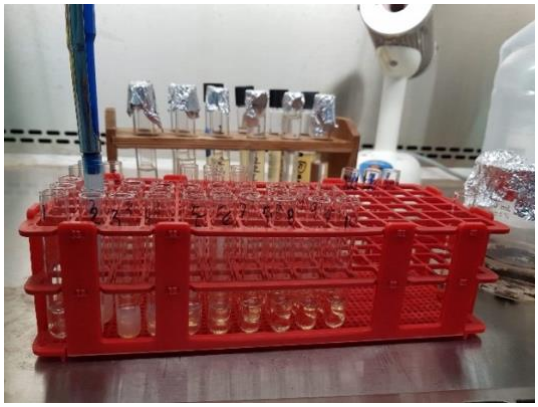


Figure 6. MIC determination through macrobroth dilution.

The tube with the lowest concentration was subcultured by streaking 10µL of the contents of the tube with no visible growth or turbidity and the first tube with visible growth on Mueller-Hinton agar plates (Figure 7). The plates were incubated for 24 hours at 37ºC. The concentration of the tube producing one colony or no colony at all on Mueller-Hinton plates was reported as, MBC [18].

Figure 7. MBC determination.

RESULTS AND DISCUSSION

Aqueous fruit extract preparation

Fifty grams of unripe *A. bunius* fruit were placed into a blender along with the addition of distilled water and 200 mL aqueous extract was collected and used throughout the process. The appearance of the extract (Figure 8) showed clear yellow brown with watery consistency which is similar to the yellowish fruit extract obtained in the study.

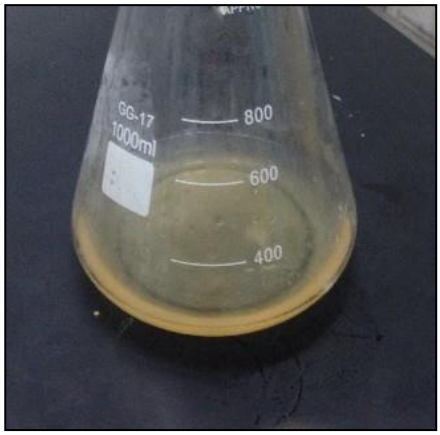


Figure 8. *A. bunius* extract.

Synthesis of nanoparticles silver

Addition of *A. bunius* unripe fruit extract into silver nitrate resulted into a yellowish white solution. Upon exposure to sunlight for an hour, the solution turned from yellowish brown into reddish brown indicating reduction of silver ion as shown in Figure 9. This most likely due to silver nitrate's sensitivity to light wherein once exposed, it will start to hydrolyze.

This color change is also supported by the studies Sulaiman et al. [15] wherein synthesis of silver nanoparticles were carried out with increasing sunlight exposure, resulting to the change in color from yellow to reddish brown due to the excitation of surface plasmon resonance in the solution.

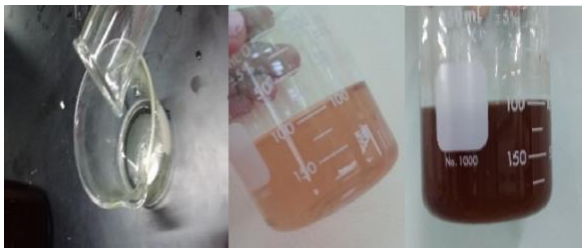


Figure 9. Color change in the synthesis of Ag NPs

UV-visible (UV-Vis) Spectroscopy

UV-Vis was used as a screening to identify the presence of nanoparticles through the peak absorbance. It was used to determine the structure and the optical properties of metal nanoparticles. It was generally recognized that UV-Vis spectroscopy could be used to examine size and shape of nanoparticles in the aqueous suspension. Based on the results of this study, absorption spectra of Ag NPs formed in the reaction media had an absorbance peak at 445 nm scanned at 200-800 nm range as shown in Figure 10.

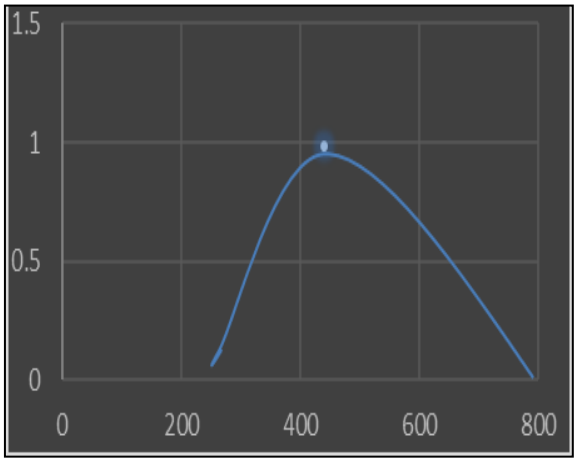


Figure 10. Ultra-visible spectroscopy result of Ag NP

According to the study of Sulaiman et al. [15], Ag metal has the capability of being soft, white and lustrous. It exhibited electrical, thermal conductivity and even reflectivity. A spherical Ag NPs having a maximum absorbance between 420 to 450nm. This indicates that pure silver nanoparticle was produced.

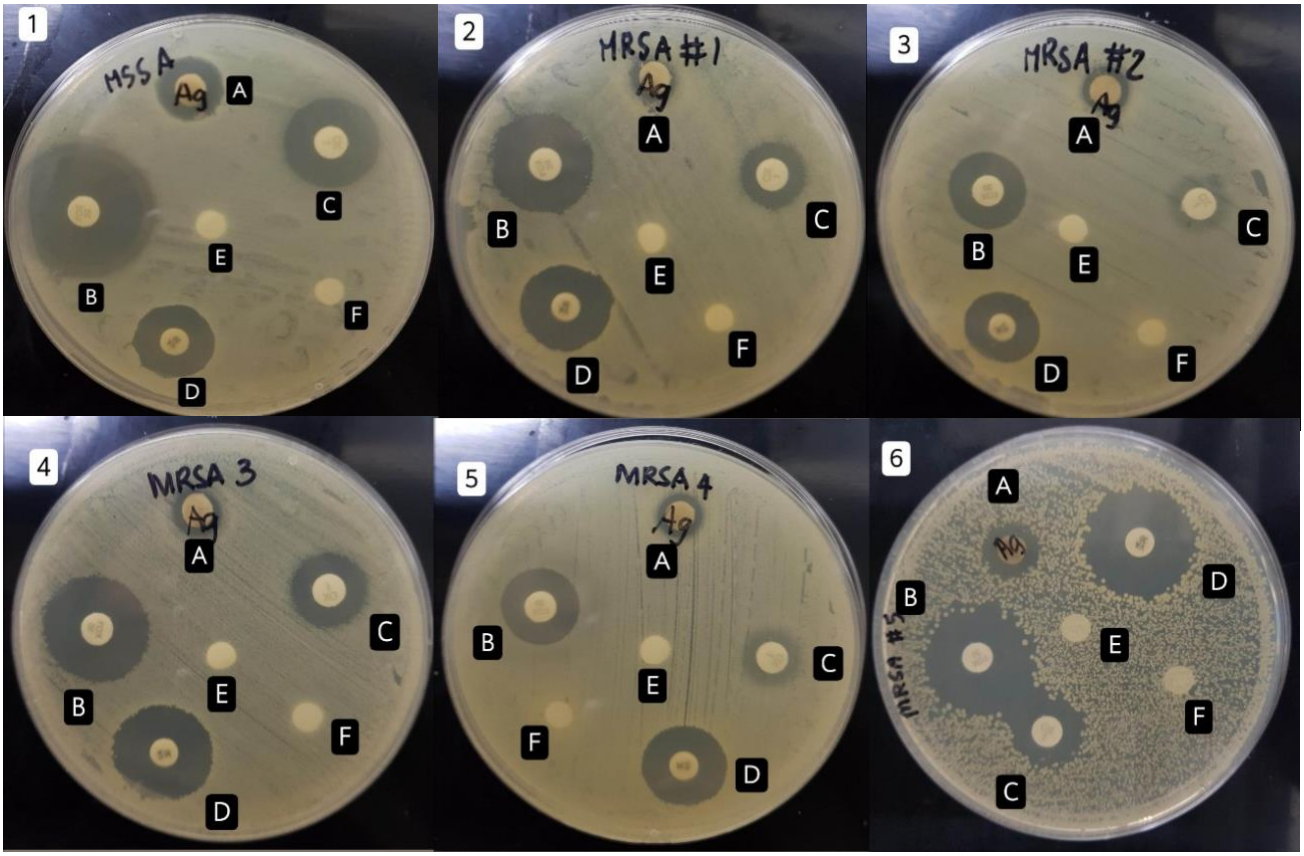
Disc diffusion method

The antibacterial activity of Ag NPs produced was tested against ATCC 29213 Methicillin-susceptible *S. aureus* and five isolates of Methicillin-resistant *S. aureus.*

Table 1 shows the zones of inhibition of Ag NPs, standard antibiotics, distilled H2O, and sterile blank discs against MSSA and MRSA. The positive controls, namely Oxacillin (Ox), Vancomycin (Va) and Cefoxitin (CFX) exhibited zones of inhibition of 19 mm, 18 mm and 29 mm, respectively against MSSA (other consideration all susceptible based on CLSI standards) while Ag NPs with a concentration of 10 uL exhibited a zone of inhibition of 14 mm. The negative and vehicle control, including a sterile blank disk and one loaded with distilled water, on the other hand, showed no visible zone of inhibition. This shows that Ag NPs have lesser activity than the standards used to inhibit MSSA. Based on the study of J. Li et al. (2014) , oxacillin remains the first-line agents in treating infections caused by MSSA.

Table 1
Results of Disc Diffusion Method Zones of Inhibition

	Ag NPs (10 uL)	Cefoxitin (CFX)	Oxacillin (Oxa)	Vancomycin (Va)	Distilled H_2O	Sterile Blank Disc
MSSA	14 mm	29 mm	19 mm	18 mm	6 mm	6 mm
MRSA #1	7 mm	21 mm	13 mm	20 mm	6 mm	6 mm
MRSA #2	8 mm	16 mm	12 mm	17 mm	6 mm	6 mm
MRSA #3	10 mm	22 mm	15 mm	23 mm	6 mm	6 mm
MRSA #4	9 mm	17 mm	12 mm	18 mm	6 mm	6 mm
MRSA #5	10 mm	20 mm	11 mm	21 mm	6 mm	6 mm



Legend: (A) Silver nanoparticles, (B) Cefoxitin, (C) Oxacillin, (D) Vancomycin, (E) Distilled H_2O, (F) Sterile blank Disc

Figure 11. Visible clear zone produced by unripe fruit extract mediated Ag NPs against MSSA/MRSA (1) Methicillin-susceptible *Staphylococcus aureus* ATCC 29213 (2-6) Clinical isolates: Methicillin-resistant *S. aureus*

For MRSA, the result of Ag NPs against the first isolate exhibited a zone of inhibition of 7 mm. Ox, Va and CFX showed zones of inhibition with measurements of 13 mm, 20 mm and 21 mm, respectively. For the second isolate, a zone of inhibition of 8 mm was observed around the Ag NPs-loaded disk. The positive controls which included Ox, Va and CFX exhibited zones of inhibition with measurements of 12 mm, 17 mm and 16 mm, respectively.

The Ag NPs against the third isolate exhibited a zone of inhibition of 10 mm and same results were also observed against the fifth isolate. Ox, Va and CFX showed zones of inhibition with measurements of 15 mm, 23 mm and 22 mm, respectively while the standard antibiotics against the fifth isolate showed zones of inhibition with measurements of 11 mm, 21 mm and 20 mm, respectively.

For the fourth isolate, a zone of inhibition of 9 mm was observed around the Ag NPs-loaded disk. The positive controls: Ox, Va and CFX exhibited zones of inhibition with measurements of 12 mm, 18 mm and 17 mm, respectively. All the sterile blank disks (negative control) and ones loaded with distilled water (vehicle control) exhibited no visible zone of inhibition against MRSA. The antibacterial activity showed in both first and second isolates were lower than the obtained result of 9 mm while the Ag NPs against the third and fifth isolate showed almost similar results as with the size of 10-13 mm. The zone of inhibition exhibited by the Ag NPs against the fourth isolate were closest to the zones size of 9- 12 mm.

Moreover, it was observed that Ag NPs were also able to inhibit MRSA with 7mm, 8mm, 10mm, 9mm and 10mm zones of inhibiton for the five isolates. Though there are inhibitions observed, the measurements were lower than those produced using CFX, Oxa and Va. This shows that these three antibiotics can inhibit MRSA better than AgNPs. According to the study of Adhikari et al., [19], vancomycin was the drug of choice for treating infections caused by MRSA. This implicates that the nanoparticles synthesized in this study are also active against MRSA although, when compared to the standard drugs, they exhibit inefficient and lesser inhibitory activity against the bacterial pathogens. For further evaluation, MIC and MBC were also performed.

Table 2. Results of Ag NPs tube dilution test after being incubated at 37 ºC for 24 hours. Minimum Inhibitory Effect of Silver Nanoparticles

Tube	Concentration (mg/mL)	MSSA	MRSA				
			1	2	3	4	5
1	97.08138	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
2	48.54069	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
3	24.27035	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
4	12.13517	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
5	6.06759	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
6	3.03379	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
7	1.5169	Clear	Turbid	Turbid	Turbid	Turbid	Turbid
8	0.758448281	Clear	Clear	Clear	Clear	Clear	Clear
9	0.379224141	Clear	Clear	Clear	Clear	Clear	Clear
10	0.18961207	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
11	Negative Control	Turbid			Turbid		
12	Media Control	Clear			Clear		
13	Positive Control	Clear			Clear		

The MIC was determined using Mueller- Hinton broth (MHB) with serial two-fold dilutions of each nanoparticle. In this study, the controls used for both MSSA and MRSA included a positive control using 1 mL of MHB and 1 mL of Vancomycin, a media control having only 2 mL of MHB and a negative control which contained 1 mL of MHB and 1 mL of bacterial suspension. All were incubated at 37ºC for 18-24 hours.

In the macrobroth dilution, the negative control tube showed turbidity indicating growth of bacteria whereas, the media and positive control tubes showed clearing indicating absence or inhibition of bacteria. Upon streaking in Mueller- Hinton agar for confirmation, bacterial growth was evident in the negative control and absence of growth was observed in the media and positive controls. The MIC is indicated by the lowest concentration of antimicrobial agents that visually inhibited the growth of microorganisms. The MIC was examined through the visual turbidity of the tubes both before and after incubation to confirm its efficacy against the tested bacteria [20].

The result of Ag NPs tube dilution is shown in Table 2. For MSSA, clearing of tubes was observed in tubes 7 to 9 with concentrations of 0.38 to 1.52mg/mL. Clearing against five isolates of MRSA was consistently observed in tubes 8 and 9 having concentrations of 0.38 to 0.76 mg/mL. White precipitates were evident in tubes 1 to 6 of MSSA and tubes 1 to 7 of the five MRSA isolates as presented in Figure 12. Upon further inspection, it was found out that the MHB composition contained HCl hydrolyzed casein, serving as an amino acid supply producing Chloride salts as part of its production process which then reacted with the Silver Nitrate. Thus, the reason behind the turbidity of the tubes in MIC testing was most likely due to the formation of Silver Chloride precipitate.

Same results were also observed in the study of Vijayakumar et al., [21] which showed that silver is heavy metal as it tends to form white precipitate.

Thus, silver nanoparticles obtained in this study exhibited antimicrobial activity against MSSA and MRSA.

After determining the MIC of Ag-NPs, aliquots of 10 µl from all tubes were seeded in MHA agar plates. They were then incubated at 37ºC for 24 hours. Results of MBC of Ag NPs are shown in Table 3. The MBC was observed for the presence or absence of bacterial growth in agar plates both before and after incubation [20]. Absence of growth against MSSA was evident from plates seeded with bacterial suspension from tubes 1 to 8. Results are shown in Figure 13.

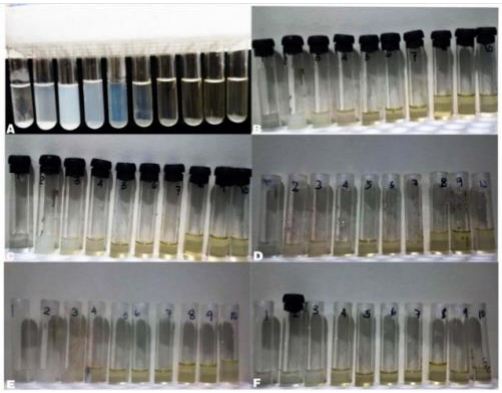


Figure 12. MIC determination of Ag NPs using macrobroth dilution showing clear and turbid solutions against (A) MSSA ATCC 29213 (B) MRSA#1, (C) MRSA#2,(D) MRSA #3, (E) MRSA #4 and, (F) MRSA #5

Table 3. Results of Ag NPs on Mueller-Hinton agar plates after being incubated at 37 ºC for 24 hours. Minimum Bactericidal Effect of Silver Nanoparticles

Tube	Concentration (mg/mL)	MSSA	MRSA				
			1	2	3	4	5
1	97.08138	-	-	-	-	-	-
2	48.54069	-	-	-	-	-	-
3	24.27035	-	-	-	-	-	-
4	12.13517	-	-	-	-	-	-
5	6.06759	-	-	-	-	-	-
6	3.03379	-	-	-	-	-	-
7	1.5169	-	-	-	-	-	-
8	0.758448281	-	-	-	-	-	-
9	0.379224141	+	-	-	-	-	-
10	0.18961207	+	+	+	+	+	+
11	Negative Control	+			+		
12	Media Control	-			-		
13	Positive Control	-			-		

Legend: *Positive sign (+) indicates bacterial growth. Negative sign (-) indicates absence of growth.*

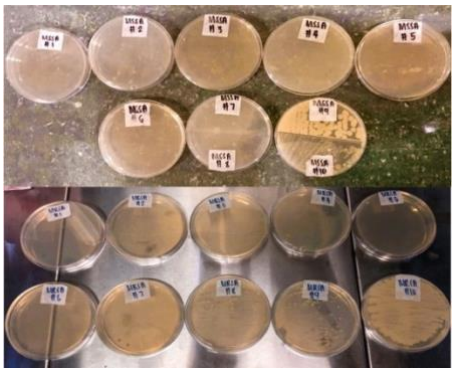


Figure 13. MHA plates containing 10µl of after being incubated at 37 ºC for 18-24 hours

Growth of MSSA was observed from tube 9 and 10 with tube 8 having a concentration of 0.76 mg/mL was noted as the lowest concentration of Ag NPs to inhibit the growth of MSSA. For the five MRSA isolates, tubes 1 to 9 showed absence of growth while tube 10 was observed with growth of MRSA. Tube 9 with a concentration of 0.38 mg/mL was noted as the lowest concentration of Ag-NPs to inhibit growth of MRSA. The MBC results was complementary to MIC, where MIC test demonstrates lowest level of Ag NPs that greatly inhibits growth of MRSA and MSSA. MBC demonstrates the lowest level of Ag NPs resulting to microbial death.

In the MIC test, turbidity in tubes number 1 to 6 in MSSA and tubes number 1 to 7 for MRSA were observed due to Silver Chloride precipitate formation. The same

tubes where then subcultured in MHA agar and MBC to ensure that it was indeed silver nanoparticles. It was noted that concentrations of 0.38 mg/mL and 0.76 mg/mL have an absence of visible growth in MSSA and MRSA respectively.

In this study, MIC and MBC of Ag NPs against MRSA and MSSA was determined by macrodilution method. It was found to be effective at 0.38 mg/mL and 0.76 mg/mL. According to the study of Krishnan et al. (2015), it demonstrated 5 mg/mL of Ag NPs concentration which is higher than the results of this study. This variation was due to different methodologies that were used to prepare Ag NPs. Nevertheless, it was confirmed in this study that Ag NPs have antimicrobial property which correlates to the results of preliminary screening of the disc diffusion methods.

Furthermore, according to Almayahi and Alhusseini [22], different naturally occurring stabilizing and environmentally benign reducing agents have been making headways recently with regards to the biosynthesis of metallic nanoparticles. These include polysaccharides, plant extracts, as well as bacteria and fungus.

For instance, Vilchis-Nestor et al. [23] employed green tea extract as an alternative reducing and stabilizing agent to synthesize gold and silver nanoparticles. Kalishwaralal et al., [24], on the other hand, reduced Ag NPs by using Bacillus licheniformis' culture's supernatant. The resulting nanoparticles were deemed highly stable, having to use a nonpathogenic kind of bacterium.

As antibacterial agents, Ag NPs have shown promising reports and researches. One of it being its application as a disinfecting agent on medical devices as shown in the work of Bosetti et al. (2002) wherein its antibacterial behavior has been noted effective on implantable medical devices. Moreover, the textile industry has also even pursued the incorporation of silver nanoparticles onto fabrics. Lee et al. [25] study showed silver nanocomposite cotton fibers exhibiting anti-bacterial activity against Escherichia coli. In the study performed by Mohammed and Senthil [26] the Enterococcal isolates showed sensitivity on both synthesized gold and silver nanoparticles. A directly proportional relationship was also observed between the dosage employed and their anti- bacterial activity levels.

In relation to these previous works, the synthesized silver nanoparticles in this study exhibited a positive response in manifesting its antimicrobial activity toward MSSA and MRSA isolates, thus further proving the importance of nanoparticles in the field of medicine overall.

CONCLUSION AND RECOMMENDATION

Unripe A. bunius extract can reduce silver nanoparticles. The synthesized silver nanoparticles can inhibit MSSA and MRSA, however, their inhibitory activity is lesser than that produced by the standard antibiotics. Therefore, bio-reduced metal nanoparticles using A. bunius can be used as a potential, natural antimicrobial agent against inhibiting Staphylococcus aureus infections.

Future studies on the antimicrobial effects of metal nanoparticles bio-reduced by unripe A. bunius (bignay) fruit extract is highly recommended. Use of other nanoparticles like gold, copper and zinc and various parts like ripe fruits, leaves, bark, and roots of bignay tree are recommended for future testing.

Researchers also recommend increasing the concentration of the synthesized nanoparticles for stronger activity against inhibiting bacteria. Use of other methods in the characterization of the metal nanoparticles like Scanning Electron Microscopy, Transmission Electron Microscopy, and X-ray Diffraction Assay are likely recommended as well.

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