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BIOFORMULATION OF SILVER NANOPARTICLES FROM BETEL LEAF (PIPER BETEL) EXTRACT AND ITS ANTIOXIDANT, CYTOTOXIC AND ANTIBACTERIAL PROPERTIES

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ABSTRACT

Silver nanoparticles have diverse qualities with significant applications. Amidst the challenge of growing antimicrobial resistance, incorporating these nanoparticles into drug delivery systems could offer solutions. Thus, their extensive production holds promise, particularly in healthcare. This experimental research aimed to unveil the potential of Betel leaf (Piper betel) extracts to formulate Silver Nanoparticles and their antibacterial, antioxidant, and cytotoxic properties. UV-VIS Spectrophotometry was utilized to quantify the bioformulated AgNPs. The presence of biosynthesized silver nanoparticles was verified by the color change in all five varying concentrations of solution from greenish to dark brown. The decrease in bacterial growth rate becomes noticeable as the concentration of Ag nanoparticles rises. AgNPs in Sample 5 (higher concentration) exhibited the highest antibacterial activity for Staphylococcus aureus (11.25 mm and Escherichia coli (11.20 mm)—both partially active. The lowest activity was observed in Sample 1(lowest concentration) for both S. aureus and E. coli (10.16 mm). The result of the study shows that at 1000 ppm for the first three hours, no shrimps died. On the other hand, in the succeeding hours, the mortality rate increases. The study revealed that silver nanoparticles can be synthesized in a simple method using betel leaf extract. It is a straightforward, effective, and environmentally friendly process.

Keywords: Green Synthesis, Betel Leaf, Nanotechnology, Antimicrobial Resistance, Lethality Assay, Disk Diffusion Assay

1. INTRODUCTION

Nanotechnology has progressed significantly in research over the last century. Nanoparticles (NPs) are categorized based on their shape, size, and chemical properties. Different methods have been used to study other physical and chemical characteristics of NPs. These include X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), infrared (IR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), Brunauer–Emmett–Teller (BET), and

particle size analysis Parray et al. (2021). The field of nanotechnology has grown rapidly in this century. Zhang et al. (2016) noted that new progress in nanoscience and nanotechnology has completely transformed how we detect, treat, and stop different illnesses across all aspects of human existence. Silver nanoparticles (AgNPs) are particularly important and captivating among various types of tiny metal particles used in medical applications. Silver nanoparticles account for more than 23% of all nano products. Silver nanoparticles possess a wide variety of properties that makes them valuable for different purposes. In a world threatened by the rise of antimicrobial resistance, the use of silver nanoparticles in drug delivery systems may help solve this emerging problem. Therefore, their large-scale synthesis has the potential of being extremely useful, especially in healthcare.

Metals like gold (Au), silver (Ag), palladium (Pd), zinc (Zn), copper (Cu), and iron (Fe) are chosen due to their specific structures and shapes. These metals exhibit distinct d–d transition features and strong localized surface plasmon resonance (LSPR) effects. This has been discussed in studies by Khan et al. (2017), Mohan et al. (2014), and Dreaden et al. (2012). Silver nanoparticles find significant applications in medicine and healthcare. Unlike regular medicines, nanoparticles appear to work well with the body. This compatibility allows for precise treatment, making therapies more effective and less harmful. As a result, silver nanoparticles hold great promise as treatments. They possess various properties like fighting microbes, viruses, and cancer, aiding in clot dissolution, preventing blood clotting, and assisting in diagnosis and imaging.

In the world of green chemistry, an emerging field that aims to make chemical processes more eco-friendly, silver nanoparticles are considered safe for humans and animals when used in small amounts. They also don't cause considerable damage to the environment which sets them apart from other metal nanoparticles. Several studies have suggested different ways for the eco-friendly synthesis of metal nanoparticles, like using microorganisms and plant extracts, that are safer such that of Makarov et al. (2014) and Prasad et al. (2018). Currently, there's an increasing demand to create nanoparticles in ways that are kinder to the environment, avoiding harmful chemicals during the production process, as discussed by Whitesides (2003). From the perspective of the replacement of hazardous and non-renewable chemicals, the utilization of plant extract as a reductor in the synthesis of metal nanoparticles is an emerging method that is widely developed Ahmed et al. (2016) . Hence exploring sources of biosynthesized silver nanoparticles from indigenous plants would be a great way to discover and utilize the potential of ethnographic plants in the Philippines.

One of these ethnographic plants is the Betel leaf (Piper betel) which is renowned for its extensive healing properties. Phytochemical studies on piper betel leaves showed the presence of alkaloids, tannins, carbohydrates, amino acids, and steroidal components. The biomolecules present create its effects like being a laxative and anti-parasitic substance. Piper betel freshens breath, aids the heart, and has many roles like fighting fungi, protecting cells, aiding digestion, and more. It also affects the brain, cools fever, fights cancer, reduces inflammation, and influences the immune system and blood clotting. Parts of the piper betel used are the stem, roots, leaves, stalks, and fruits. The numerous curative properties of the betel leaf extracts include anti-diabetic, anti-mutagenic, anti-inflammatory, antibacterial, antioxidative, and anti-hemolytic. Staphylococcus aureus and Escherichia coli have stopped their activity against betel leaf extracted in water Chakraborty and Shah (2011), and ethanol Mahfuzul Hoque et al. (2011). Manigauha et al. (2009) noted that the methanolic extracts from betel leaves exhibit abilities to reduce, and scavenge DPPH radicals, counteract superoxide anions, and degrade deoxyribose.

The Betel leaves are also reported to possess the ability to fight cancer, especially against tobacco carcinogens Chang et al. (2002) , Wu et al. (2004). Research by Rai et al. (2011) also revealed that betel leaves can hinder the development of oral cancer, stomach cancer, and breast cancer.

Piper betel L. or betel vine also called "Ikmo" in Pangasinan and "Gawed" in Cordilleran provinces has about 100 varieties in the world. It is a creeping plant that is common among East African and Southeast Asian countries. Use of Betel leaf in the Philippines is common to tribes in the Cordillera Administrative Region in their practice of "Moma"/" Mama"/" Nganga" etc. or chewing of betel nut.

This research aimed to explore the potential of the betel leaf in the bioformulation of silver nanoparticles as an antibacterial, antioxidant, and cytotoxic agent. This will be relevant in the development of new drugs formulated from endemic plants in the Philippines.

2. MATERIALS AND METHODS

Research Environment

The research focused on the bioformulation of silver nanoparticles (AgNPs) from betel leaf extract. The antibacterial, cytotoxicity, and antibacterial properties were tested. Samples were collected at Hapid, Lamut, Ifugao and were brought to the SMU Center for Natural Sciences for experimentation.

Methods and Procedures

1) Plant extract preparation

- The collected betel leaves from Hapid, Lamut, Ifugao were brought to the SMU Center for Natural Sciences and oven-dried at 70 degrees Celsius for 3 days.
- The dried sample was blended to pulverize the leaves and was soaked in 500ml of 95% ethyl alcohol.
- After 72 hours, the mixture was filtered using a glass funnel and cotton to remove the undissolved leaf particles. The mixture was then put into a water bath for the extract to be evaporated at 40 degrees Celsius until it had a syrup consistency.

2) Preparation of Silver Nitrate

A 95:5 ratio was used, and 3.4g of silver nitrate was combined with 100 mL of distilled water. The solution was stirred continuously. Another 100 mL of distilled water was combined with 100 mL of silver nitrate solution.

3) Bioformulation of Silver Nanoparticles (AgNPs) from Silver Nitrate

In an Erlenmeyer flask, 38 mL of $AgNO_3$ and 2 mL of leaf extract were combined and stirred continuously using a glass stirring rod. This was stirred until a chemical reaction occurred. This was observed once the silver nanoparticles change color from yellow to dark brown.

4) High-speed centrifuge

The 95% $AgNO_3$ and 5% leaf extract were placed in a centrifuge tube measuring 10 mL each. For 25 minutes, the solution was placed in a centrifuge at 6000 rpm. This was to separate the solid and liquid parts of the solution in layers and then further separated them through decantation.

5) Percentage Yield

The solid precipitates of the solution were measured using an electronic weighing scale to obtain the percentage yield in the 95% $AgNO_3$ and 5% leaf extract.

6) UV-VIS Spectrophotometry

In a solution (1:4 diluted water) of the reaction mixture, the UV-Vis spectrum was used to measure the bioformulated AgNPs. It was compared with 4.5mL of distilled water as a blank.

7) Antibacterial activity

To test for the antibacterial activity of the sample, the disc diffusion method was used. Nutrient Agar (NA) was used to screen the in vitro antibacterial activity of the sample.

- **Preparation of Culture, Media, Positive Control, Negative Control, and Extract**

Nutrient agar and *gulaman* bar were cooked. This was mixed with 1000mL tap water and prepared in three Erlenmeyer flasks. The bacteria culture was incubated at 35 ℃ for 24 hours. After the extraction, it was filtered using cotton balls and placed in a beaker. Filter disc of 6mm diameter was immersed in the positive control: 0.25g/L Streptomycin, negative control: Ethyl alcohol and Betel leaf extract for 24 hrs.

- **Dispensing media in a petri dish**

Nutrient agar was allowed to cool down before it was dispensed into the bottom of the petri dish. Then it was gently rotated to evenly distribute the medium without any splash over the sides. The tissue paper was used to wipe any moisture on the cover of the petri dish, as there should be no moisture on the cover to avoid any water droplets on the colony formed in the medium.

- **Swabbing of bacteria**

The inoculum suspension was swabbed uniformly with the bacteria namely *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). It was dried for 10 minutes. For 24 hours, the filter paper discs were soaked in the positive control, negative control, and leaf extract and then placed on the surface of the medium and the compound. This was allowed to diffuse for 5 minutes.

- **Incubation**

The setups were kept for incubation at 37℃ for 24 hours. The plates were kept for measuring the Zone of Inhibition. Inhibition zones were found around the disc and were measured using a Vernier caliper. This was done in three replicates.

8) DPPH Radical Scavenging Assay

The concentrated betel leaf extract was used to make a stock solution. The aliquot was taken to 500 ppm dilution and 500 Catechin as control (1mg/mL). One mL of prepared stock solution was mixed with four mL of 0.1 Mm DPPH in a separate plastic cuvette. This procedure was done in triplicate. Absorbance readings were monitored using a UV-Vis spectrophotometer.

9) Brine Shrimp Lethality Assay

Artificial seawater was used in hatching brine shrimps into their larval stage. Forty-eight (48) hours were allowed to pass before confirming the petri dish for the hatched eggs. Confirming the hatchlings, the extract was prepared by getting an aliquot of the extract. 3 mL of the extract was mixed with 3 mL of artificial seawater. The extract was then mixed and placed in replicate vials.

Using two-fold dilution, 15 vials were prepared with 1000ppm, 500ppm, 250ppm, 125ppm, and 65 ppm extract and AgNPs of Betel leaf. The lethality was measured every three hours for 24 hours. In each vial, 10 brine shrimps were added per concentration. This was to measure the mortality of the brine shrimps in each vial per concentration at a given time. The mortality rate was manually counted using a magnifying glass and a flashlight.

3. RESULTS AND DISCUSSIONS

3.1. VISUAL CHARACTERIZATION ON THE FORMATION OF SILVER NANOPARTICLES

The utilization of plants to create silver nanoparticles has gained interest lately. This approach is quick, environmentally friendly, safe, and cost-effective. It offers a one-step method for biosynthesis, as explained by Pal et al. (2019). UV-VIS Spectrophotometry was used to measure the bioformulated AgNPs. After combining the varying concentrations of the crude extract and silver nitrate solution, the formulation of biosynthesized silver nanoparticles is expected. The presence of biosynthesized silver nanoparticles was verified by the color change in all five varying concentrations of solution from greenish to dark brown. This is in accord with the study done by Samuel et al. (2020) who reported the same color changes due to the reduction of Ag+ ions. Change into dark brown color was also observed by Lagashetty et al. (2019) in their biosynthesis of silver and gold using the same sample.

During the synthesis, the color change was observable with constant stirring of the mixture. Solutions were continuously mixed until there was the formation of cloudy particles suspended in the mixture. The color changes were observed within 10 minutes of constant stirring.

3.2. UV-VIS

To detect the visual characteristic and spectrum absorbance of the biosynthesized AgNPs, a UV-vis spectrophotometer was used. Due to surface plasmon resonance (SPR) the AgNPs are expected to give a peak at a particular wavelength which will confirm its presence. The tables below show the absorbance values of the silver AgNPs in the different concentrations.

Table 1

Table 1 Average Absorbance of the Synthesized AgNPs at 1:49 Concentration

Silver Nanoparticles (1:49)								
TIME	**Initial**				**30 mins**			
NM	S_1	S_2	S_3	Ave	S_1	S_2	S_3	Ave
200	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
300	3.22	4.00	3.22	3.48	3.00	3.52	3.00	3.17
400	2.54	3.52	2.54	2.86	2.54	2.15	2.54	2.41
500	1.08	1.93	1.08	1.36	1.08	1.71	1.08	1.29
600	0.58	1.23	0.58	0.80	0.58	1.27	0.58	0.81
700	0.49	1.04	0.49	0.67	0.49	1.35	0.49	0.78

Table 2

Table 2 Average Absorbance of the Synthesized AgNPs at 2:48 Concentration

Silver Nanoparticles (2:48)								
TIME	**Initial**				**30 mins**			
NM	S_1	S_2	S_3	Ave	S_1	S_2	S_3	Ave
200	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
300	3.00	3.00	3.00	3.00	4.00	4.00	4.00	4.00
400	3.70	4.00	3.70	3.80	4.00	4.00	4.00	4.00
500	1.87	1.77	1.87	1.84	1.96	2.74	1.96	2.22
600	1.14	1.97	1.14	1.42	1.17	1.76	1.17	1.37
700	0.99	2.09	0.99	1.36	1.12	1.50	1.12	1.25

Table 3

Table 3 Average Absorbance of the Synthesized AgNPs at 3:47 Concentration

Silver Nanoparticles (3:47)								
TIME	**Initial**				**30 mins**			
NM	S_1	S_2	S_3	Ave	S_1	S_2	S_3	Ave
200	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
300	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
400	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
500	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
600	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
700	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00

Table 4

Table 4 Average Absorbance of the Synthesized AgNPs at 4:46 Concentration

Silver Nanoparticles (4:46)								
TIME	**Initial**				**30 mins**			
NM	S_1	S_2	S_3	Ave	S_1	S_2	S_3	Ave
200	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
300	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
400	3.00	4.00	3.00	3.33	3.00	3.00	3.00	3.00
500	2.52	2.24	2.49	2.42	3.00	3.00	3.00	3.00
600	1.58	1.40	1.57	1.52	3.00	3.00	3.00	3.00
700	1.37	1.23	1.35	1.32	3.00	3.00	3.00	3.00

Table 5

Average Absorbance of the Synthesized AgNPs at 5:45 Concentration

Silver Nanoparticles (5:45)								
TIME	**Initial**				**30 minutes**			
NM	**S_1**	**S_2**	**S_3**	**Ave**	**S_1**	**S_2**	**S_3**	**Ave**
200	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
300	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
400	3.00	4.00	3.00	3.00	3.00	3.00	3.00	3.00
500	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
600	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
700	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00

Table 6

Table 5 Summary of Average Absorbance of the Synthesized Nanoparticles (AgNPs) in the Different Concentrations

Silver Nanoparticles										
TIME	**Initial**					**30 minutes**				
Conc.	1:49	2:48	3:47	4:46	5:45	1:49	2:48	3:47	4:46	5:45
NM										
200	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
300	3.48	3.00	3.00	3.00	3.00	3.17	4.00	3.00	3.00	3.00
400	2.86	3.80	3.00	3.33	3.33	2.41	4.00	3.00	3.00	3.00
500	1.36	1.84	3.00	2.42	3.00	1.29	2.22	3.00	3.00	3.00
600	0.80	1.42	3.00	1.52	3.00	0.81	1.37	3.00	3.00	3.00
700	0.67	1.36	3.00	1.32	3.00	0.78	1.25	3.00	3.00	3.00

Table 7

Table 6 Average Absorbance of the Distilled Water as a Blank

Distilled water								
TIME	**Initial**				**30 mins**			
NM	S_1	S_2	S_3	Ave	S_1	S_2	S_3	Ave
200	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.01
300	0.05	0.06	0.05	0.05	0.04	0.05	0.04	0.04
400	0.04	0.05	0.04	0.04	0.04	0.05	0.04	0.04
500	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
600	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04
700	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04

Five different concentrations of the solution were prepared and triplicates were made from each concentration. Distilled water as a blank was first placed in the 5ml cuvette for UV-Vis's spectroscopy. Then it was followed by the different concentrations of the solution which were tested for initial characterization and were tested again after 30 minutes. Initial confirmation of the presence of AgNPs was done by the change in visual color. The summary table on the averages of the different concentrations shows that AgNPs are characterized based on their highest absorbance in the spectrophotometer. The UV-Vis showed a mean absorbance of 3.80 at 400nm for the initial and 4.00 at 300-400nm at 30 minutes of characterization. This is similar to that of Nguyen et. al (2021) with peaks in the wavelength of 400-450 nm. The study of Lagashetty et al. (2019) has a close result with the SPR at 430 nm indicating the presence of silver nanoparticles. A higher absorbance peak was observed by Samuel et. al at 460 nm.

3.3. ANTIBACTERIAL ACTIVITY

The synthesized AgNPs were screened in vitro for their antibacterial activity against *E. Coli* and *S. aureus* by agar disc diffusion method. The results obtained are presented in the following table.

Table 8

Table 7Antibacterial Assay of Betel Leaf AgNPs

Plant Sample	TEST ORGANISM
	Mean Zone Inhibition Obtained in mm

	Staphylococcus aureus	*Escherichia coli*
Betel leaf (Raw Extract)	14.65 mm	12.57 mm
Positive Control	28.95 mm	31.74 mm
Negative Control	6 mm	6 mm

Betel leaf obtained a mean inhibition zone of 14.65 mm against *S. aureus* and 12.57 mm against *E. coli.* The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

Table 9

Table 8 Antibacterial Assay of Betel Leaf AgNPs in Different Concentrations

Plant Sample	TEST ORGANISM Mean Zone Inhibition Obtained in mm	
	Staphylococcus aureus	*Escherichia coli*
Sample 1 (1:49)	10.16 mm	10.16 mm
Sample 2 (2:48)	10.78 mm	10.19 mm
Sample 3 (3:47)	10.82 mm	11.06 mm
Sample 4 (4:46)	11.05 mm	11.08 mm
Sample 5 (5:45)	11.25 mm	11.20 mm
Positive Control	32.46 mm	31.47 mm
Negative Control	6 mm	6 mm

Meropenem and clindamycin were used as a positive control. The reduction of bacterial growth rate on increasing the concentration of Ag nanoparticles is evident. AgNPs in Sample 5 (higher concentration) exhibited the highest antibacterial activity for *Staphylococcus aureus* (11.25 mm and *Escherichia coli* (11.20 mm)—both partially active. The lowest activity was observed in Sample 1(lowest concentration) for both *S. aureus* and *E. coli* (10.16 mm). In general, biosynthesized AgNPs ranges from partially active to inactive against *S. aureus* and *E. coli* with increasing antibacterial property with increasing level of concentration. It may appear in the table that the raw extract has a higher antibacterial property than the biosynthesized AgNPs which is in contrast with a comparative study done by Nguyen et. al (2021) but it is also important to take into consideration the concentration used by the researcher in this study as compared with other studies which are relatively lower. The results however can be compared to the study of Foronda & Cajucom (2023) wherein their samples exhibited higher zones of inhibition than their extracting agent and negative control.

3.4. CYTOTOXIC PROPERTIES (BSLA)

A brine shrimp lethality assay was used to determine the cytotoxic properties of the sample. The lethality was measured every three hours for 24 hours. In each vial, 10 brine shrimps were added per concentration. This is to measure the mortality of the brine shrimps in each vial per concentration in a given time Foronda & Cajucom (2023). The results of the test are shown in the following tables below.

Table 10

Table 9 Brine Shrimp Lethality Assay Result (Raw Leaf Extract)

	Percent Mortality Rate (%) Raw Leaf Extract							
Concentration	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs
1000ppm	13.3	56.7	83.3	90	100	100	100	100

500ppm	6.7	26.7	70	70	93.3	100	100	100
250ppm	6.7	13.3	40	50	66.7	76.7	100	100
125ppm	0	3.3	16.67	20	30	46.7	100	100
62.5ppm	0	0	10	20	26.7	43.3	100	100
LC_{50}	3,648.1	884.9	464.04	387.7	218.9	6.7	Toxic	Toxic

Table 11

Table 10 Brine Shrimp Lethality Assay Result (1:49)

	Percent Mortality Rate (%) 1:49							
Concentration	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs
1000ppm	0	0	3.3	13.3	23.3	43.3	53.3	63.3
500ppm	0	0	0	6.7	16.7	30	33.3	43.3
250ppm	0	0	0	0	6.7	20	23.3	33.3
125ppm	0	0	0	0	0	10	10	23.3
62.5ppm	0	0	0	0	0	3.3	6.7	13.3
LC_{50}	Non-toxic	Non-toxic	Non-toxic	3,394.3	1939.4	1099.9	893.5	685.4

Table 12

Table 11 Brine Shrimp Lethality Assay Result (2:48)

	Percent Mortality Rate (%) 2:48							
Concentration	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs
1000ppm	3.3	3.3	6.7	33.3	43.3	56.7	70	80
500ppm	0	0	0	16.7	26.7	43.3	56.7	66.7
250ppm	0	0	0	3.3	13.3	23.3	33.3	43.3
125ppm	0	0	0	0	3.3	10	16.7	23.3
62.5ppm	0	0	0	0	0	3.3	6.7	10
LC_{50}	Non-toxic	Non-toxic	7,238.8	1,423.7	1,094.5	790.04	590.4	462.6

Table 13

Table 12 Brine Shrimp Lethality Assay Result (3:47)

	Percent Mortality Rate (%) 3:47							
Concentration	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs
1000ppm	10	10	13.3	43.3	56.7	66.7	73.33	76.6
500ppm	0	0	3.3	23.3	40	53.3	63.3	70
250ppm	0	0	0	6.7	20	33.3	40	50
125ppm	0	0	0	3.3	13.3	30	40	46.7
62.5ppm	0	0	0	0	0	3.3	10	16.7
LC_{50}	4,960.3	4,960.3	3,561.8	1,123.5	809.7	609	470.4	349.1

Table 14

Table 13 Brine Shrimp Lethality Assay Result (4:46)

	Percent Mortality Rate (%) 4:46							
Concentration	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs

1000ppm	6.7	10	23.3	66.7	83.3	93.3	100	100
500ppm	0	0	10	50	63.3	76.6	86.7	96.7
250ppm	0	0	6.7	23.3	40	53.3	66.7	86.7
125ppm	0	0	3.3	16.7	26.7	33.3	46.7	60
62.5ppm	0	0	0	13.3	13.3	23.3	26.6	33.3
LC_{50}	7,238.8	4,960.3	2,154.4	656.2	453.2	305.1	169.7	Toxic

Table 15

Table 14 Brine Shrimp Lethality Assay Result (5:45)

Percent Mortality Rate (%) 5:45								
Concentration	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs
1000ppm	6.7	16.7	66.7	86.7	100	100	100	100
500ppm	0	3.3	46.7	60	80	93.3	100	100
250ppm	0	0	16.7	30	53.3	63.3	83.3	93.3
125ppm	0	0	6.7	20	30	50	66.7	80
62.5ppm	0	0	3.3	16.7	26.7	33.3	50	63.3
LC_{50}	7,238.8	2,915.6	699.3	481.9	287.1	120.1	Toxic	Toxic

The tables show that at 1000 ppm for the first three hours, no shrimps died. On the other hand, in the succeeding hours, the mortality rate increases. According to Meyer et al., if the crude plant extract has an LC50 value of less than 1000 µg/mL, it is toxic (active) while if it is greater than 1000 µg/mL, it is non-toxic (inactive). The LC50 for the first three hours is greater than 1000 ppm thus the silver nanoparticles from the crude extract of betel leaf are non-toxic but as time increases, the LC50 decreases to less than 1000 ppm resulting in more toxic (active) AgNPs. The 4:46 concentration of the biosynthesized AgNPs showed toxicity at the 24th hour being the lowest concentration achieving toxicity. At 4:45 concentration, brine shrimps were completely eliminated at the 21st hour being the fastest-acting sample.

3.5. ANTIOXIDANT ACTIVITY

The antioxidant activity of the aqueous extract and plant AgNPs was evaluated using DPPH scavenging. 15 vials with 1000ppm, 500ppm, 250ppm, 125ppm, and 65 ppm extract and AgNPs of Betel leaf were tested. Using the DPPH assay, the biosynthesized AgNPs in plant extracts were compared to the raw extract and the Cathechin as a positive control. The result of the test is shown below.

Table 16

Table 16 DPPH Assay Result

Sample Description	Absorbance Reading			Mean Absorbance	% Radical Scavenging Activity (RSA)
	1	2	3		
Raw Extract	0.170	0.161	0.167	0.166	81.39%
49:01	0.275	0.280	0.277	0.277	68.94%
48:02	0.233	0.241	0.194	0.223	75.00%
47:03	0.193	0.188	0.179	0.187	79.04%
46:04	0.192	0.193	0.201	0.195	78.14%
45:05	0.197	0.207	0.194	0.199	77.69%
Cathechin (control)	0.121	0.146	0.121	0.129	85.50%

Highest RSA achieved is 81.39% with the raw extract. This reveals that biosynthesized AgNPs in the plant samples yield lower RSA. All samples have lower RSA compared to the positive control ranging from 68 – 80%. Nevertheless, the sample yields good results with RSA higher than 50% and is very close to the result of the positive control. The results showed that the extract and biosynthesized AgNPs are still effective as an antioxidant agent.

4. CONCLUSION AND RECOMMENDATION

This study determined that silver nanoparticles can be synthesized using betel leaf extract. It is confirmed via visual characterization as observed by the changing color of the sample from green to dark brown. Further confirmation of the biosynthesized AgNPs is shown by the results of the UV-vis spectroscopy with maximum absorbance reading at around 300-400 nm. The raw extract of betel leaf has manifested higher effectivity in terms of antibacterial, antioxidant, and cytotoxic properties. Still, the biosynthesized silver nanoparticles showed antibacterial and cytotoxic properties, more so with their antioxidant property very close to the positive control. The biosynthesis of AgNPs in betel leaf still holds possibilities that can be explored more.

This work may be further developed with a more in-depth characterization and applications of silver nanoparticles. The researchers recommend characterizing silver nanoparticles to a higher level of characterization and a wider range of concentrations should be used to yield significant results. Other properties of biosynthesized silver nanoparticles may even be explored.

CONFLICT OF INTERESTS

None.

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