

PHYSICOCHEMICAL PROPERTIES AND ANTIBACTERIAL ACTIVITY OF BIOSYNTHEZIZED SILVER NANOPARTICLES FROM *Melothria pendula* Linn. (PIPINONG-GUBAT) LEAF EXTRACT

ABSTRACT

Nanoparticles have all kinds of unexpected benefits in different fields and industry. For this reason, the demand for nanoparticles is increasing at overwhelming rate the increased demand resulted to increase in production. Conventional method in synthesizing nanoparticles have been reported to be capital intensive, inefficient in materials and energy use. Because of this, researchers are leaning to biological method in synthesizing NPs. In this study, aqueous leaf extract of *Melothria pendula* Linn. (Pipinong-gubat) was used to biosynthesized silver nanoparticles (AgNPs) and the biosynthesized nanoparticle underwent physicochemical and antibacterial analysis. Physical and chemical properties of leaf extract have been determine having a black color, pleasant odor, boiling point of 94.3°C, density of 0.94g/mL, pH of 8 slightly basic and polar in both water and ethanol and non-polar in hexane. Secondary metabolites that are present in leaf extract are alkaloids, saponins and terpenoids. Having a coloration of yellowish-brown, the synthesized AgNPs was characterized using UV-VIS Spectrophotometer that the resulting peak confirm the formation of AgNps, Fourier Transform Infrared (FTIR) determined the functional group that was present and Scanning Electron Microscopy (SEM) determined the surface morphology that shows AgNps in agglomeration state at a magnification of (a) 1000 k (b) 2000 k (c) 3000 k (d) 4000 k and a diameter of (a)100 um (b)30 um (c)30 um (d) 20 um. In terms with its antibacterial result, it was found out to be effective against *S. aureus* and *E. coli*. It has a clear zone of inhibition of 5mm in *S. aureus* and 3.67mm in *E.coli*. Furthermore, statistical treatment shows that there is no significant in antibacterial activity between positive control (Chloramphenicol) and sample (AgNPs). This implies that the sample could be a substitute to the market as antibacterial agent against *S. aureus* and *E. coli*.

Keywords: *Melothria pendula* Linn., biosynthesize, silver nanoparticles, antibacterial effect

I. INTRODUCTION

Melothria pendula Linn. (Pipinong-gubat) is a perennial prostrate or climbing vine with thin and smooth stems and coiled tendrils, growing to a length of six feet or more. Leaves are alternate, toothed, shallowly or deeply five-lobed, reaching a length of 5 to 7 centimeters wide. Fruit is a tiny, green to black, smooth, and watermelon-like berry, oblong-elliptic, 10 to 19 millimeters long, about 12 millimeters in diameter, with white spots when young, dangling at the end of the pedicel. This plant thrives in wet compost soils, with slightly acidic or neutral pH. It appears mainly on the outskirts of forests of jungles, in meadows, near ponds, riverbanks, and on bushes in open fields, under full sun or light shade. But it can be also found growing on poor, dry soils, in urban areas, sometimes from the cracks in concrete pavement. It can withstand strong heat, and when older and stronger, even quite strong drought. But it is very frost-sensitive. It is often hated as an obnoxious weed rapidly overgrowing cultivated plants, especially crops as it grows fast where the soil is well watered and fertilized, and when the temperature is high. And it is seldom grown on purpose.

Melothria pendula Linn. plant is identified to be in the Cucurbitaceae plant family. *Melothria pendula* Linn. is native to Mexico and Central America, and get naturalized in the south and east of the USA, Brazil some other North American Countries, and many regions in Southeast Asia and China.

Lira (1998) stated that *Melothria pendula* Linn. has been mentioned as a wild species of the Cucurbitaceae family in Mexico that continues without being studied to its full extent. According to the “chemical-bromatologic” analysis of this plant carried out in the present work, this vegetal species under study constitutes a source of water, vitamins, minerals, and amazingly, also proteins. The fruits of this plant, despite their reduced size, have a pleasant, sweet flavor and are edible for humans. Besides, its foliage is given to livestock as forage. For this reason, this “wild cucumber” could be an additional nutritional alternative for men and animals. It was stated that this plant contains 12.6% protein, 16.30% fiber, and 56.8% carbohydrates.

In Mexico, an infusion of the fruit is used as a tonic for anemia, and boiled fruits are used as a remedy for heart disease. Infusion of the aerial parts of the plant is taken in the treatment of diabetes for its hypoglycemic effect and is used as a remedy against gastritis, calculus, and sores. The crushed fresh plant is used for snake bites applied topically against rash and hemorrhoids, and in general as an anti-inflammatory.

Conventionally, nanoparticles like silver nanoparticles are synthesized using either physical or chemical methods, which include sol process, micelle, chemical precipitation, hydrothermal method, pyrolysis, and chemical vapor deposition. Using this conventional method is capital intensive and inefficient in materials and energy use. Biological methods have emerged as an alternative to the conventional methods for the synthesis of nanoparticles. Using this method makes nanoparticles more biocompatible and environmentally friendly and its process is more cost-effective than conventional methods. In the biological method, the researcher usually uses bacteria, fungal species, and plant extract. Using bacteria and fungi to synthesize AgNPs has been reported to accumulate AgNps intracellularly meaning it takes a longer reaction time and demands subsequent extraction and recovery steps. On the contrary, plant extract-mediated synthesis always takes place extracellularly, as a result, it has a shorter reaction time than microbial synthesis. And this process is suitable for large-scale synthesis of the nanoparticle.

It is also proven that when metal oxides are reduced to nano size, properties are enhanced due to the large surface area wherein particles can interact more than their bulk counterpart (Dagalea, Lim, 2021). Such example of an enhanced property is the ability to kill microbes. Nanosized can rupture the cell of the bacteria due to their very small size. Thus, this study is beneficial to our community to develop products that have antibacterial property on it. Hence, this study used *Melothria pendula* Linn. leaf extract in synthesizing silver nanoparticles. The plant is native to the Philippines, particularly in the province of Northern Samar. The synthesized AgNps have undergone characterization through UV-vis spectrophotometer for wavelength spectra, Fourier Transform Infrared (FTIR) spectroscopy for structural determination, and Scanning Electron Microscopy (SEM) for surface morphology. After that, biosynthesized silver nanoparticles underwent an antibacterial test against *Escherichia coli* and *Staphylococcus aureus*.

II. METHODOLOGY

The *Melothria pendula* Linn. (Pipinong-gubat) leaves were collected from Las Navas, Northern Samar. The said leaves were washed with distilled water and dried in the drying oven. The researcher used an electrically powered grinding machine to powder the dried leaves. The said powdered leaves were boiled in deionized water and filtered for synthesizing silver nanoparticles.

Determination of Physical Properties

Boiling Point. About two (2) mL of *Melothria pendula* Linn. (Pipinong-gubat) leaf extract was placed in a test tube. The test tube with the extract was heated in bath oil, and when it starts to boil the temperature was recorded. This process was repeated thrice to have an accurate result.

Color. The color of *Melothria pendula* Linn. (Pipinong-gubat) leaf extract was observed by the researcher and five respondents using their sense of sight. Having five respondents is the way of getting an accurate result. A majority is a rule to be noticed in this test.

Density. The extract of *Melothria pendula* Linn. (Pipinong-gubat) leaf extract was poured into a graduated cylinder, then its volume was recorded and weight in analytical balance. The density of the *Melothria pendula* Linn. (Pipinong-gubat) leaf extract was calculated by the mass divided by its volume.

Odor. The odor of *Melothria pendula* Linn. (Pipinong-gubat) leaf extract was observed by the researcher and five respondents using their sense of smell.

pH. The *Melothria pendula* Linn. (Pipinong-gubat) leaf extract was determined by using pH indicator strips. About 10 mL of the sample leaf extract was placed in a beaker and three pH indicator strips were dipped into the extract. After the three pH indicator strips dried the color pattern was then analyzed and the pH of the extract was obtained.

Solubility. Three solvents were used namely; hexane, water, and ethanol. Nine (9) test tubes were used, each containing 2 mL of the plant sample extract. These 9 test tubes were divided into three (3) groups, each group having two milliliters of solvent to be poured in. The 9 test tubes were then observed for one minute to determine the solubility of the plant sample extract in three different solvents, and the results were recorded. If the solute is miscible in both ethanol and water the solute is said to be polar, on the other hand, if the solute is miscible to hexane, it is non-polar.

Determination of Secondary Metabolites (Guevara, 2005)

Test for Alkaloids. About 5 g of powdered leaves were soaked with the right amount of ethanol and stood for 30 minutes before filtration. After filtration, the ethanolic extract was then dried in a boiling water bath and the dried extract was then washed with the right amount of 2M HCl and filtered. In this test, Dragendorff's reagent, Mayer's reagent, and Hager's reagent (Picric acid solution) were used in determining the presence of alkaloids. The filtrate was then separated into three groups each group consisting of 3 test tubes with 2mL filtrate. The first group added Dragendorff's reagent with 2-3 drops, the second group added Mayer's reagent with 2-3 drops, and the third group added Hager's reagent with 2-3 drops. A positive result was indicated by the appearance of orange precipitate in Dragendorff's, white or cream-like precipitate in Mayer's, and yellow precipitate in Hager's reagent.

Test for Saponins. Froth test for saponins was used. Two grams (2 g) of a powdered leaf were boiled in 20 mL of distilled water in a water bath and filtered in the solution. 2.5 mL of filtrate was then added to 10mL distilled water in a test tube. The solution was shaken vigorously for about 30 seconds and allowed to stand for 30 minutes. The indication of the presence of saponin, and honeycomb froth is still present after 30 minutes.

Test for Terpenoids. Based on the Salkouski test, about 5 mL of the sample leaf extract was mixed with 2 mL of chloroform, and 3 mL of concentrated H₂SO₄ was carefully added along the side of the test tube to a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids.

Preparation of Silver Nitrate Solution

The following method in synthesizing silver nanoparticles was taken from Ramteke (2012) with a little modification. An accurately weighed 0.017 grams of silver nitrate was dissolved in 90mL of de-ionized water and stored in a bottle for further use. The bottle with the solution was covered with aluminum foil to avoid degradation of the silver nitrate.

Biosynthesis of Silver Nanoparticles

About 10 grams of powdered leaves were boiled in 100mL deionized water for 5-10 minutes and then let stand to cool down. After it cool down, it was then filtered and about 10mL of the filtrate was added to 100mL silver nitrate solution and let stand at room temperature for 24-48hrs. The change in color from yellow/orange to a dark brown or cloudy solution indicated the formation of silver nanoparticles in the plant extract.

Characterization of Biosynthesized Silver Nanoparticles

UV-vis Spectroscopy Analysis. The researcher in this study used the Ultra-violet Visible Spectrophotometer to analyze the absorbance peak of the silver nanoparticles.

FTIR Spectroscopy Analysis. The researcher in this study used this technique to determine the functional groups that are responsible for the synthesis and obtain the complete spectrum of the silver nanoparticles.

Scanning Electron Microscopy (SEM) Analysis. The researcher in this study used this technique to determine the size and shape of the synthesized silver nanoparticles.

Antibacterial Screening (Brensis, 2018)

a) Source of Test Organism

Pure isolates of *Escherichia coli* and *Staphylococcus aureus* that are used in the study were obtained from the Department of Science and Technology, Tacloban.

b) Preparation of Bacterial Subculture in Nutrient Agar

About 7 grams of Mueller Hinton Agar was suspended in 243 mL sterilized distilled water. Wherein, the Mueller Hinton Agar is the common type of agar media used in antibacterial screening. The mixture was boiled with frequent agitation to completely dissolve the media and it was then sterilized by the pressure cooker with a temperature of 121°C and

pressure of 15 psi for 15 minutes. The media was allowed to cool at about 50°C then it was aseptically poured into each petri dish and allowed to solidify.

c) Preparation of the Filter Paper Disc for the Sample, Positive and Negative Control

A sterilized filter paper was cut into a round disk shape using a puncher. The number of filter paper discs is dependent on the number of trials in the study. The filter paper discs were then soaked in AgNps, Chloramphenicol (dissolved in water), and distilled water.

Determination of Antibacterial Activity

A modified Kirby Bauer (1966) antibacterial sensitivity test was used to determine the antibacterial activity of synthesized AgNPs against *E.coli* and *S. aureus*. Pure isolates of *E. coli* and *S. aureus* were aseptically harvested into the surface of nutrient agar plates with the use of a sterilized cotton swab. The soaked filter paper discs were then placed on the surface of nutrient agar plates with sterile pick-up forceps. The inoculated plates were incubated for 24 hours at a temperature of 30°C. After 24 hours, the plates were inspected for the presence of any clear zone of inhibition around sample discs. A clear zone around the sample disc indicates that the organism was susceptible to the chemical agent present in the discs, hence, inhibiting the growth. The absence of any clear zone suggests that the organisms were resistant to the chemical agent present in the disc.

III. RESULTS AND DISCUSSION

This study aimed to determine the physicochemical properties and antibacterial activity of biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) aqueous leaf extract. Specifically, it aimed to verify the physical properties of the leaf extract in terms of color, odor, density, pH, and solubility. The secondary metabolites determined in this study were: alkaloids, saponins, and terpenoids. The researcher also characterized the synthesized silver nanoparticles by using UV-VIS, Surface morphology, and FTIR analysis.

Physical properties

Boiling Point, about two (2) mL of *Melothria pendula* Linn. (Pipinong-gubat) leaf extract is placed in a test tube. The test tube with the extract is heated in bath oil, and when the extract starts to boil the temperature is recorded. It has an average boiling point of 94.3°C. **Color and odor** were determined by a panel of evaluators. There were color and odor samples shown to evaluators. The majority of evaluators said that the aqueous leaf extract of the sample has black color and pleasant odor. The **density** was determined by weighing (5 mL) of the extract and then dividing the weight in grams by the volume in mL. After the computation, the researcher found out that the aqueous leaf extract is 0.94g/mL. The **solubility** of the sample leaf extract was determined by using water, hexane, and ethanol as solvents. The selection of solvents was based on polarity. Water and ethanol are polar while hexane is non-polar. The **pH** was determined using pH indicator strips by pouring 10 mL of leaf extract and dipping the pH indicator strips into the extract and analyzing the change of its color pattern. After analyzing the change in color pattern, the researcher found out that the aqueous leaf extract is slightly acidic with a pH level of 8. All of the physical properties are discussed in Table 1.

Table 1. Summary Results of the Physical Properties of *Melothria pendula* Linn. (Pipinong-gubat) Leaf Extract

Physical Properties	Sample Extract	Interpretation
Boiling Point	94.3 ⁰ C	Lower Boiling Point than Water
Color	Black	Black
Density	0.94 g/mL	Less Dense than water
Odor	Pleasant	Pleasant
pH	8	Slightly basic
Solubility in: Ethanol Hexane Water	Miscible Imiscible Miscible	Polar Non-polar Polar

Determination of Secondary metabolites

The secondary metabolites determined for *Melothria pendula* Linn. (Pipinong-gubat) leaf extract collected at Las Navas, Northern Samar were alkaloids, saponins, and terpenoids.

The test for **alkaloids** was done using Dragendorff's reagent, Mayer's reagent, and Picric acid solution. A positive result was formed with a white or cream-like precipitate in treating Mayer's reagent, an orange precipitate formed in treating Dragendorff's reagent, and a yellow precipitate in Hager's reagent. The *Melothria pendula* Linn. (Pipinong-gubat) leaf extract has alkaloids.

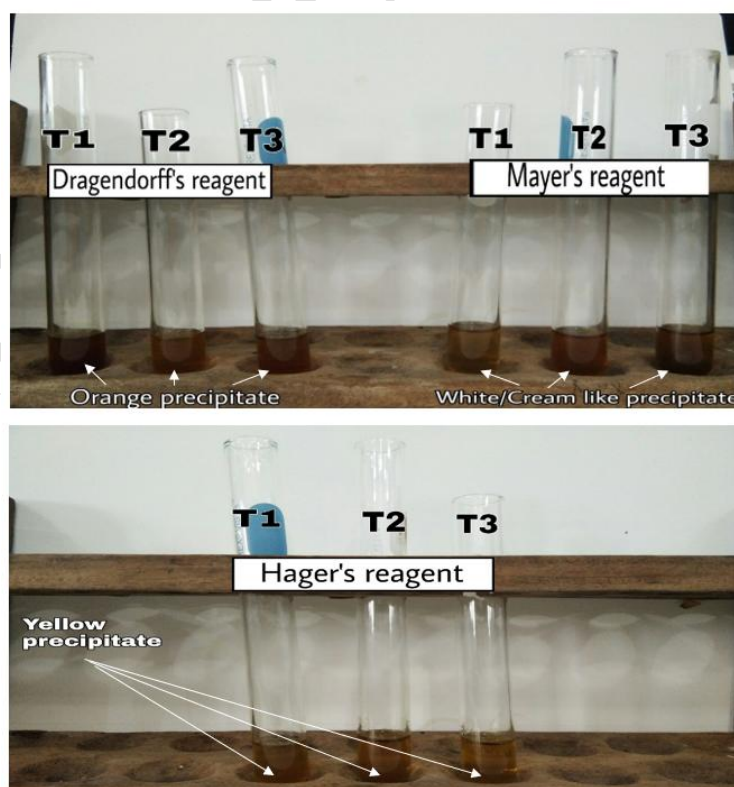


Figure 1. *Melothria pendula* Linn. (Pipinong-gubat) leaf extract test for alkaloids

Froth test for **saponins** determination was used. About 2g of a powdered leaf was boiled in 20mL of distilled water in a water bath and filtered. A 2.5mL of the filtrate was added to 10mL distilled water in a test tube. The solution was shaken vigorously for about 30 seconds and allowed to stand for 30 minutes. Honeycomb froth stays after 30 minutes and it indicated the presence of saponins. The *Melothria pendula* Linn. (pipinong gubat) leaf extract has saponins.

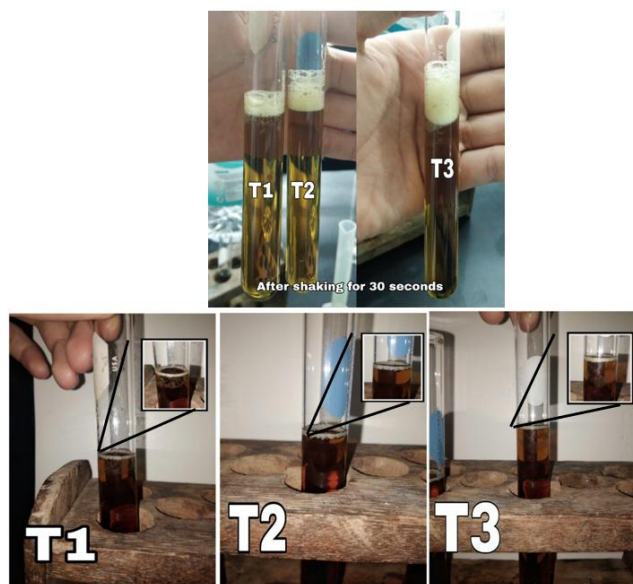


Figure 2. *Melothria pendula* Linn. (Pipinong-gubat) leaf extract test for saponins

Salkouski test for **terpenoids** was used, about 5 mL of Pipinong-gubat leaf extract was mixed with 2 mL of chloroform and 3 mL of concentrated H_2SO_4 was carefully added along the side of the test tube to a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids. Figure 3 shows reddish-brown coloration in the interface of leaf extract and chloroform. Thus, conforming to the presence of terpenoids in *Melothria pendula* Linn. (Pipinong-gubat) leaf extract.

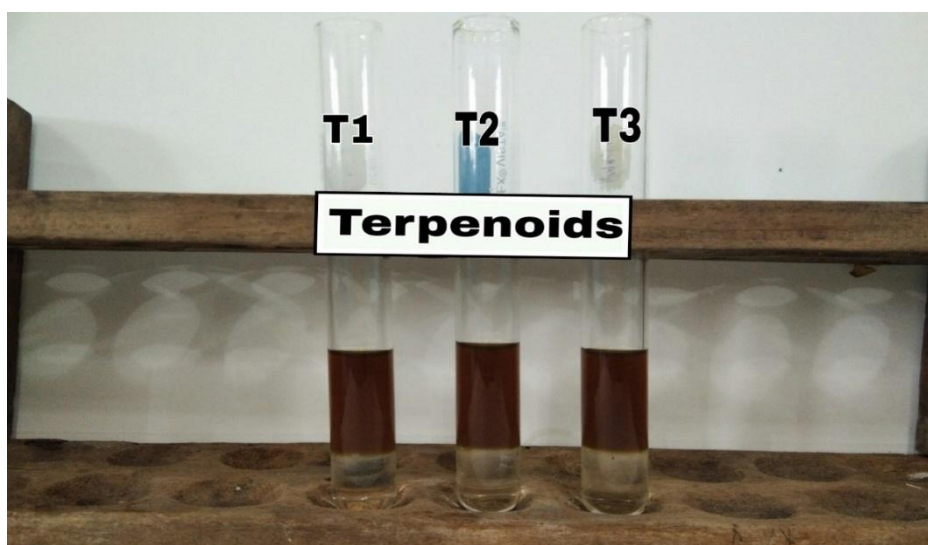


Figure 3. *Melothria pendula* Linn. (Pipinong-gubat) leaf extract test for terpenoids
 Characterization of Biosynthesized Silver Nanoparticles

UV-visible spectroscopy measures the extinction of light passing through a sample. It is a valuable tool for identifying, characterizing, and studying nanoparticles. Typical AgNPs has a λ_{max} value that is visible in the 400-800nm range and at the range of 400 nm Ag nanoparticles have a standard peak ranging from 2.531 to 2.614. In this study, the standard peak, and the sample peak at the range of 400nm are compared. Traces of Ag nanoparticles can be seen at the peaks of 1.774(trial 1), 1.814(trial 2), and 1.769 (trial 3).

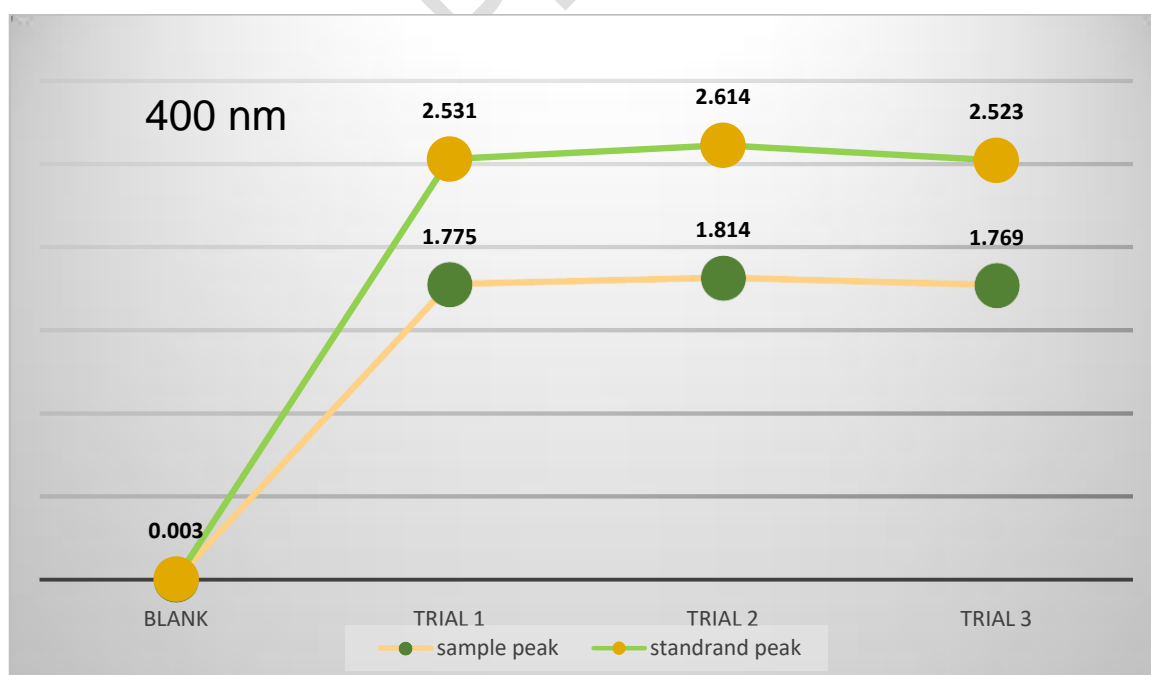


Figure 4. The absorbance of biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) leaf extract

The **FTIR** analysis helps to indicate certain functional groups that are present in the sample. The result shows that the peak with a range of 3053.32 indicates the presence of amine-type ($C \equiv N$) structures and this proves that the sample contains a variety of alkaloids. In medicine, alkaloids exhibit pharmacological activity and they have been used as a bronchodilator, cardiac stimulant, muscle relaxant, pain killer, analgesic, anti-inflammatory, anti-cancer, and antibacterial. Variations of alkaloids are being used in medicine; atropine(used as an antidote to cholinesterase inhibitors), morphine and codeine(used as narcotic analgesics and antitussive agents), colchicine(used as gout suppressant), caffeine(used as central nervous system stimulant and as an antidote to barbiturate and morphine poisoning), emetine(used in treating amebic dysentery and other protozoal infections). This plant sample could be used in different ways as to the presence of the amine group.

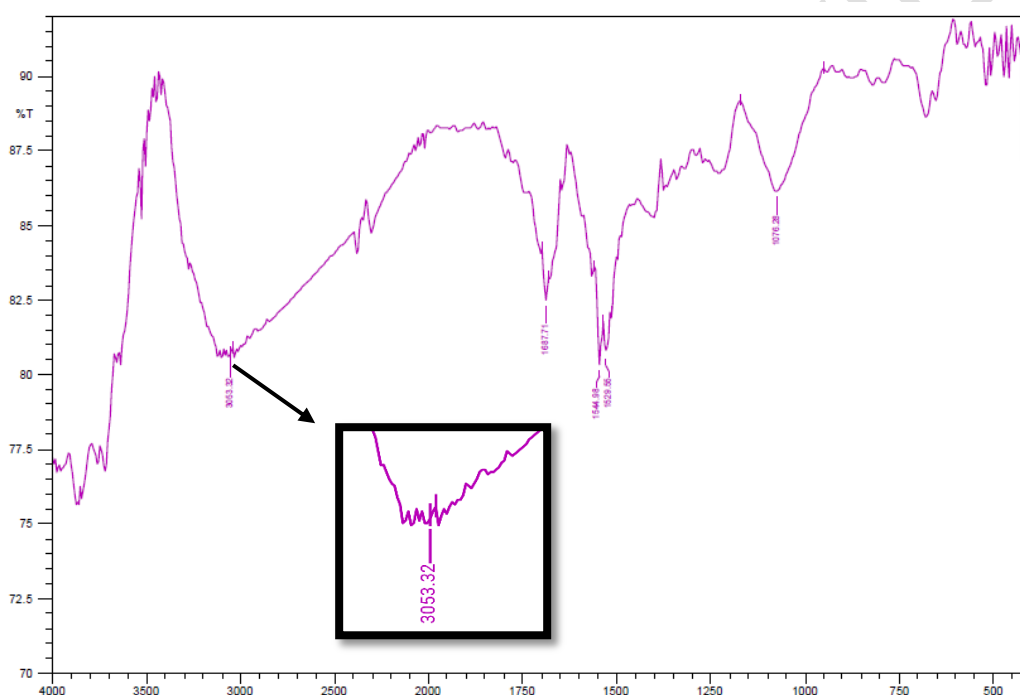


Figure 5. The FTIR spectra of biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) leaf extract

The **surface morphology** of biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) leaf extract has been evaluated by SEM analysis. The obtained images of Ag nanoparticles are shown below. The SEM images with a magnification of (a) x1000 with a diameter of 100um, (b) x2000 with a diameter of 30um, (c) x3000 with a diameter of 30um, and (d) x4000 with a diameter of 20 um show that the synthesize Ag nanoparticles are assemblage like a pile of mass due to uncontrolled agglomeration. Agglomeration is produced by nanoparticles to minimize their very high surface energy. It is advisable to conduct an immediate analysis of the surface morphology of freshly synthesized nanoparticles to avoid further agglomeration.

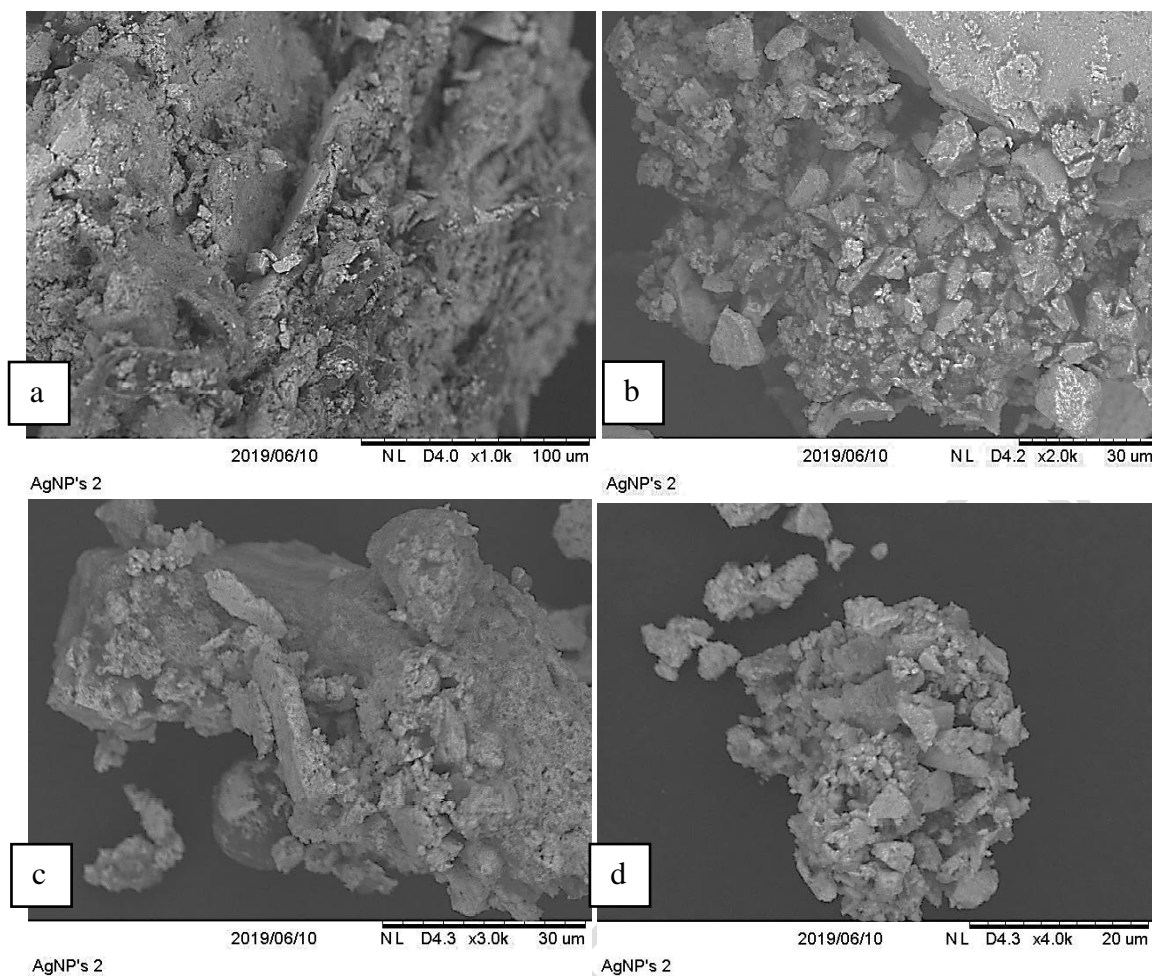


Figure 6. Surface morphology of biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) leaf extract

Results of the antibacterial activity for biosynthesized AgNps against *Staphylococcus aureus* and *Escherichia coli* are shown in the next discussion.

As shown in Table 2, after three trials, the result of biosynthesized AgNps showed antibacterial activity against both *S. aureus* showing a clear zone of inhibition of 5 mm mean in all treatments, and *E. coli* showing a zone of inhibition of 3.67 mm mean in all treatments, this implied that the sample has an antibacterial activity which inhibited the growth of *E. coli* and *S. aureus* bacteria, indicated by the presence of a clear zone of inhibition. The absence of any clear zone of inhibition suggests that the organism was resistant to the chemical agent present in the filter paper disc. On the other hand, a powder of a commercially available drug was expected to have a clear zone of inhibition and has proven to have antibiotic properties.

Table 2. Antibacterial test for the biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) leaf extract

Test Organism	TREATMENTS			
	Trials	AgNPs solution	Positive Control (Commercially available drug)	Negative Control (Distilled water)
<i>S. aureus</i>	Clear Zone of Inhibition (mm)			
	1	6	13	0
	2	4	5	0
	3	5	6	0
	Average	5	8	0
<i>E. coli</i>	1	3	4	0
	2	4	4	0
	3	4	5	0
	Average	3.67	4.33	0

Figure 7 shows the clear zone of inhibition in *S. aureus* of the sample (AgNPs), Positive control (Commercially available drug), and negative control (distilled water). Results shows 6mm(a), 4mm(b), and 5mm(c) clear zone of inhibition of the sample (AgNPs). Positive control has a 13mm(a), 5mm(b), and 6mm(c) of a clear zone of inhibition while the negative control has no clear zone of inhibition in all trials.

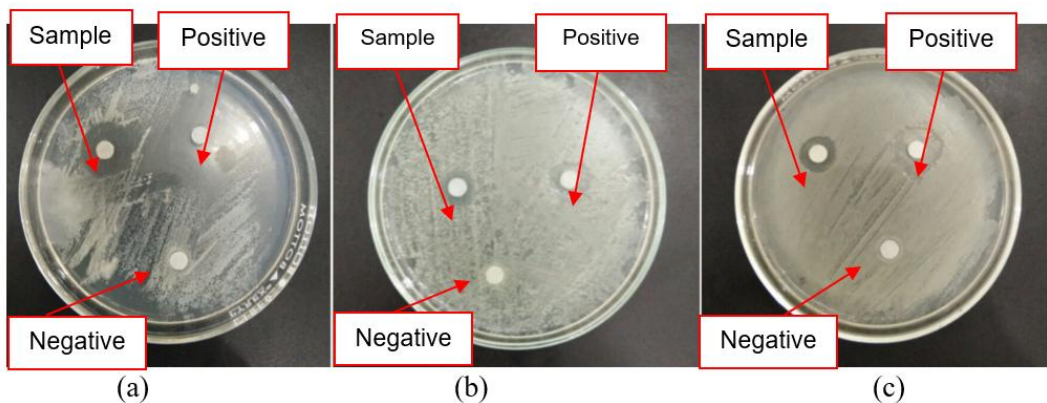


Figure 7. Biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) leaf extract against *Staphylococcus aureus*

Figure 8 shows the clear zone of inhibition in *E. coli* of the sample (AgNps), Positive control (Commercially available drug), and negative control (distilled water). Results shows 3mm(a), 4mm(b), and 4mm(c) clear zone of inhibition of the sample. Positive control has a 4mm(a), 4mm(b), and 5mm(c) of a clear zone of inhibition while the negative control has no clear zone of inhibition in all trials.

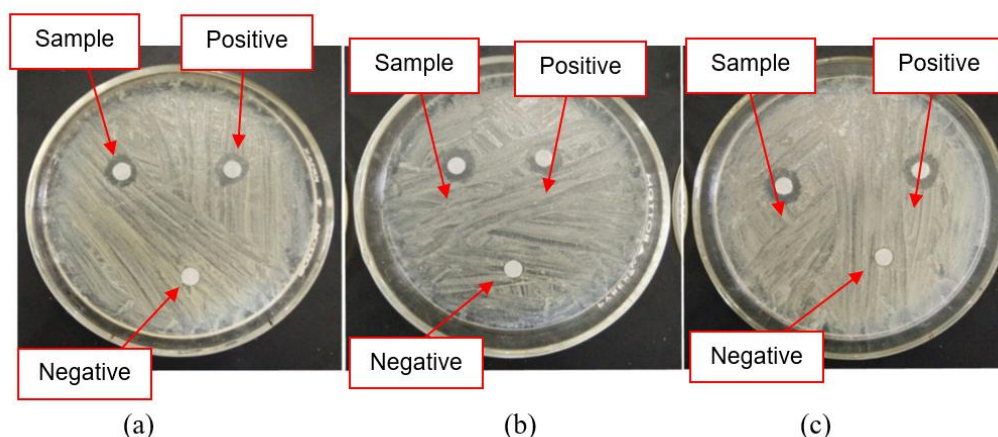


Figure 8. Biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) leaf extract against *Escherichia coli*

IV. CONCLUSION

Alkaloids, saponins, and terpenoids are the secondary metabolite that is present in *Melothria pendula* Linn. (Pipinong-gubat) leaf extract. The characterization of silver nanoparticles was done using ultraviolet-visible spectroscopy and traces of silver nanoparticles can be seen at 400nm. FTIR analysis indicates the presence of functional group amine in the synthesized AgNps which is proven to have antibacterial activity. AgNps surface morphology was determined using scanning electron microscopy (SEM) and it appeared agglomerated. And its diameter was (a)100 um in magnification of x1000, (b)30 um in x2000 magnification, (c) 30 um in x3000 magnification and (d) 20 um in x4000 magnification. Synthesized silver nanoparticles from this plant have a cloudy yellowish-brown color appearance after 2 days of reaction time. The antibacterial activity of AgNps can inhibit the growth of both *S. aureus* and *E. coli*. The synthesized AgNps has a clear zone of inhibition of 5mm in *S. aureus* and 3.67mm in *E. coli*. It is within the 2mm standard clear zone inhibition. With the promising result, biosynthesized silver nanoparticles from *Melothria pendula* Linn. (Pipinong-gubat) leaf extract could be a substitute in the market as an antibacterial agent. However, a further test must be done to properly evaluate the safety of the said new product.

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