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Synthesis of silver nanoparticles using leaf extract of *bidens pilosa* linn.: Partial characterization and evaluation of its antimicrobial activity

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Abstract: Metallic nanoparticles (MNPs) have become one of the most remarkable aspects of nanotechnology due to its wide applications especially in the field of medical industry. MNPs can be topically applied to wounds and burns to prevent infections, hence, a good source of agents having antibacterial, antifungal, and antiviral properties. In this present study, green approach of silver nanoparticles (AgNPs) synthesis was conducted using *Bidens pilosa* Linn. leaf extract. The antimicrobial activity of the synthesized AgNPs was assessed using paper-disc diffusion method against Gram-positive, Gram-negative and fungi microorganisms. Results show inhibition activity against the microorganisms, however, there is no observable inhibition trend seen as the volume of the leaf extract to silver nitrate solution increases. Furthermore, it was observed that the effectiveness of the samples to inhibit microbial growth is not dependent on the ratio of the leaf extract to silver nitrate and also with the variation of time in synthesizing AgNPs. Partial characterization of the synthesized AgNPs using ultraviolet-visible spectrophotometer exhibited an optical property as indicated by the surface plasmon resonance peaked at 413-463 nm range. Fourier transform infrared spectroscopic analysis shows that the essential functional groups responsibly reduced silver ion to atomic silver can be attributed to the presence of carboxylic acids, amines, phenols and alcohols of the leaf sample. Scanning electron microscopy-energy dispersive x-ray spectroscopy analysis of the mixture of silver nitrate and leaf extract of *B. pilosa* Linn. confirmed the presence of quasi-spherical biosynthesized silver nanoparticles with average size of 57.38 ± 2.55 nm in diameter.

Key words: Silver nanoparticles; Bacteria and fungi; Paper –disc diffusion method; Silver nitrate; Functional groups

1. Introduction

Nanoscience has recently been established as a new interdisciplinary science. It is defined as a whole knowledge on fundamental properties of nano-sized objects (Kholoud et al., 2010). The word "nano" is used to indicate one billionth of a meter or 10-9. "Nano" is a Greek word meaning extremely small (Vadlapudi et al., 2013). Metallic nanoparticles form an important aspect of nanotechnology which has been developed for their wide applications. Nanoparticles are commonly employed as drug delivery vehicles to target specific sites such as lung tissue, as well as cancer therapy and vaccinations (Park, 2014). Among metallic nanoparticles, silver nanoparticles are the most promising one. Silver has been in use for centuries for the treatment of different diseases. However, its use declined with the emergence of metallic silver (Sharma, 2009). Metallic silver in the form of silver nanoparticles has made a remarkable comeback as silver nanoparticles, with enhanced chemical and physical properties (Rai et al., 2009). Silver nanoparticles possess excellent antimicrobial, antifungal and antiviral activities. It has long been recognized as having inhibitory effect on microbes present in medical and industrial processes (Punarulselvam et al., 2012).

Nanoparticles can be synthesized using different methods. Various approaches available for the synthesis of nanoparticles include chemical, electrochemical, photochemical, Langmuir-Blodgett and biological techniques (Roy and Das, 2015). However, these methods cannot avoid the use of toxic chemicals in the synthesis of protocol (Sahayaraj et al., 2015). This situation leads to a need to develop environmentally-friendly processes through green synthesis and other biological approaches (Roy and Das, 2015).

The use of plant parts in silver nanoparticles' synthesis has gained popularity. Biological synthesis of silver nanoparticles from plants is cost-effective, eco-friendly, easily scaled up process for large scale synthesis, non-toxic, more stable and the rate of synthesis is faster than that in the case of other organisms (Kannan et al., 2014; Ramesh et al., 2014).

Bidens pilosa Linn. (Astereseae) is a plant that has been traditionally used in medicine and is widely distributed in the Philippines. Phytochemical evaluation of this plant revealed a rich phytochemical constitution among which are acetylenic and flavonoids (Borges et al., 2015). These

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phytochemicals are good reducing agents for the synthesis of silver nanoparticles.

In this study, the ability of *Bidens pilosa* Linn. to biosynthesize silver nanoparticles was determined. The biosynthesized silver nanoparticles were partially characterized and their antimicrobial activity was evaluated using paper-disc diffusion method utilizing human pathogens such as Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus, Bacillus megaterium* and *Bacillus subtilis*) and fungi (*Candida albicans*, and *Aspergillus niger*).

2. Materials and methods

2.1. Collection and preparation of leaf extract

Twenty grams of the leaves of *Bidens pilosa* Linn. Collected from the campus vicinity of the Mindanao State University-Main, Marawi, Lanao del Sur, Philippines was washed thoroughly with distilled water to remove dust particles. These were then cut into pieces and crushed using mortar and pestle. The crushed leaves were placed in a 250mL Erlenmeyer flask containing 100mL distilled water and boiled for 20 minutes. The decocted extract was allowed to cool and then filtered using Whatman No.1 filter paper. The resulting filtrate was used for the synthesis of AgNPs.

2.2. Biosynthesis of silver nanoparticles

In the synthesis of AgNPs, 0.4247 grams of silver nitrate was weighed and dissolved in 500mL distilled water to bring about 5mM $AgNO_3$ solution. 1, 5, 10, 20 and 30 mL of the leaf extract were pipetted and placed separately in a 125mL Erlenmeyer flask. To each flask, 50mL of the $AgNO_3$ solution was added and then swirled. The change in color of the solution was observed for an hour (Ghorbani et al., 2015). The same procedures were followed but this time the color change was observed after 24 and 48 hours.

2.3. Antimicrobial screening

The paper-disc diffusion method described by Guevarra (2004) was used in the determination of the antimicrobial activity of the biosynthesized AgNPs

2.4. Characterization of silver nanoparticles

After green synthesis of AgNPs, characterization is an important step to confirm its presence. The characterization of AgNPs was done using UV-Vis, FTIR, and SEM-EDX.

2.4.1. UV-Vis absorbance spectrophotometry

The optical property of the biosynthesized AgNPs after 1, 24 and 48 hours was determined using UV-Vis spectrophotometer model Lasany Double Beam LI-2800. In the analysis, the UV-Vis spectroscopic readings were recorded at a scanning speed of 300-600 nm using distilled water as blank.

2.4.2 FTIR spectroscopy

Decocted leaf extract, and decocted extract with $AgNO_3$ solution were scanned at 650-4000 cm^{-1} and analyzed using Perkin Elmer Spectrum 100 model Fourier Transformed Infrared Spectrometer to determine the possible functional groups responsible in the reduction of silver ions to silver metal.

2.4.3 SEM-EDX spectroscopy

Scanning Electron Microscopy (JOEL JSM-6510LA) equipped with an energy dispersive x-ray spectrometer was used to determine the morphology and size details of AgNPs. The particle sizing was measured using the software Image J - a cross-platform image analysis tool developed by the US National Institute of Health.

3. Results and discussion

3.1. Visual observation

To biosynthesize AgNPs, different volumes of the decocted extract of *B. pilosa* Linn. were added with 50mL of 5mM silver nitrate solution at room temperature and its color change were observed after 0, 24 and 48 hours, respectively. The change in color of the mixtures after addition of silver nitrate solution is shown in Fig. 1.

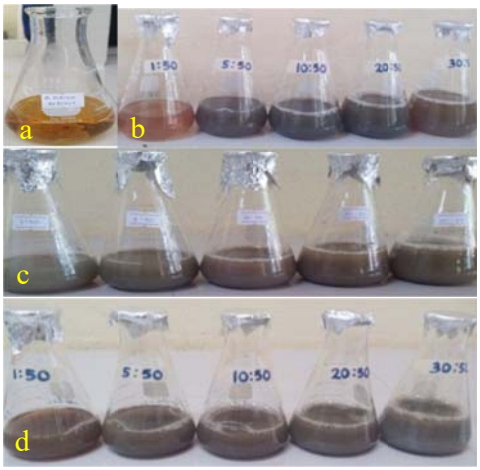

Fig. 1: Fresh leaf extract of *Bidens pilosa* Linn.(a). Color changes of different ratios of *Bidens pilosa* Linn. to silver nitrate solution after (b)1 hour (c) 24 hours (d) 48 hours.

The changes in color in the reaction vessels suggest the formation of silver nanoparticles (Kannan et al., 2014). According to Ghorbani et al. (2015), the appearances of light or dark brown color

was due to excitation of surface plasmon vibrations. This is the combination vibration of electrons of the AgNPs in resonance with the light wave. The specific oscillations depend on the shape and size of the particles. Hence, particles of different sizes show different colors.

3.2. UV-Vis analysis

UV-visible spectroscopy is one of the most widely used techniques for structural characterization of nanoparticles for it allows identification, characterization and analysis of metallic nanoparticles (Callegari, 2013) (Table 1).

Table 1: Wavelengths of the biosynthesized AgNPs at different time intervals

Leaf extract to silver nitrate ratio	Wavelength (λ_{max})	Absorbance
0 hour		
1:50	433	1.826
5:50	436	1.647
10:50	440	1.482
20:50	451	2.085
30:50	447	1.845
24 hours		
1:50	469	0.751
5:50	No peak	----
10:50	415	0.804
20:50	430	1.577
30:50	455	2.255
48 hours		
1:50	453	1.275
5:50	450	1.232
10:50	413	1.386
20:50	422	1.826
30:50	No peak	----

The maximum absorption peaks of the biosynthesized silver nanoparticles at different volume of leaf extract of *B. pilosa* Linn. is shown in Table 1. The observed UV-Vis spectrum gave rise to a single surface plasmon resonance band which is characteristic of spherical shape nanoparticles. The UV-Vis spectrum of the colloidal solutions of silver nanoparticles has absorbance peaks ranging from 413-469 nm regions measured at various hours (0, 24 and 48 hours). The observed peaks are relatively in the range of 350-550 nm which is the typical optical spectra for silver nanoparticles (Singh, 2014).

The disappearance of peak in 24 hour and 48 hours may be due to the aggregation of colloidal particles resulting to the increase in particle size which is not in nanoscale.

3.3. FTIR analysis

To determine the active functional groups present in *Bidens pilosa* Linn. leaf extract and predict their role in the synthesis of silver nanoparticles, FTIR analysis was carried out (Fig. 2 and Table 2).

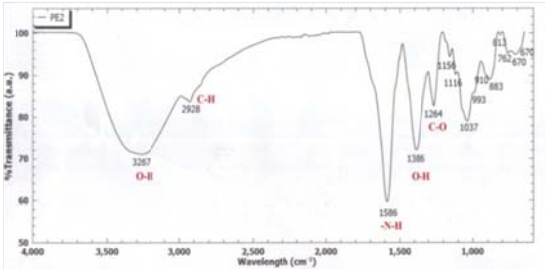

Fig. 2: FTIR spectrum of the leaf extracts of *Bidens pilosa* Linn.without silver nitrate solution

Table 2: Functional groups responsible in the reduction of silver ions to silver metals

Frequency, (cm^{-1})	Bands	Functional group
3267	O-H stretch	Carboxylic
2928	C-H stretch	Alkane
1586	N-H bending	Amine
1386	O-H bending	phenols
1264	C-O stretch	alcohols

Fig. 2 shows the absorption spectra of the leaf extract of *B. pilosa* Linn. without silver nitrate and Table 2 depicts the functional groups present in the leaf extract. The broad O-H stretching vibration at 3267 cm^{-1} is obtained for intermolecular hydrogen bonding. The aliphatic asymmetric C-H stretching vibration at 2928 cm^{-1} corresponds to a methyl group. The N-H bending vibration of amine group is detected at 1586 cm^{-1}. The O-H bending at 1386 cm^{-1} and C-O stretch at 1264 cm^{-1} corresponds to phenol and alcohol groups, respectively (Fig. 3).

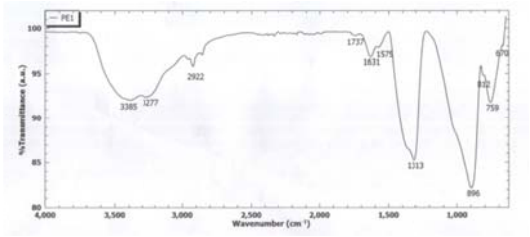

Fig. 3: FTIR spectrum of the decocted extract of *B. pilosa* Linn. with silver nitrate solution

On the other hand in Fig. 3, the bands at 3267, 1586, 1386 and 1264 cm^{-1} in Fig. 2 corresponding to O-H (carboxylic), N-H, O-H (alcohol) and C-O vibrations, respectively, were reduced (almost disappeared) in the FTIR spectrum of the decocted extract with $AgNO_3$. From these changes in the characteristic IR peaks, it can be possible that the functional groups such as carboxylic acids, alkanes, amines, phenols and alcohols which are primarily derived from heterocyclic compounds of *B. pilosa* Linn. were responsible for the reduction of silver ions to silver metal. These various functional groups are that of different water-soluble heterocyclic compounds such as tannins worked as the capping ligand during the synthesis of silver nanoparticles and the presence of oxygen atoms helped in the stabilization of nanoparticles by facilitating

absorption of heterocyclic compounds on the nanoparticles (Sahayaraj, 2014).

3.4. SEM-EDX analysis

The analysis of Scanning Electron Microscopy confirmed the presence of silver nanoparticles in the colloidal sample (1:50 ratio at 0 hour) viewed at 5000 and 1500 magnifications I shown in Fig. 4. As predicted in the UV-Vis spectrophotometry analysis, SEM micrograph of the biosynthesized silver nanoparticles from leaf extract of *B. pilosa* Linn. are quasi-spherical with an average size of 57.38 ± 2.55 nm in diameter.

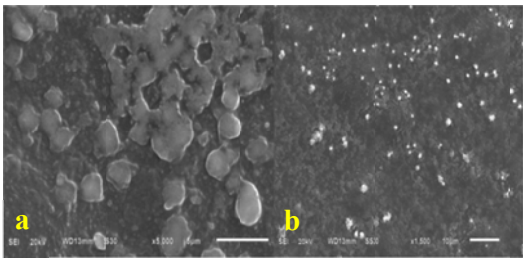

Fig. 4: SEM micrograph of biosynthesized AgNPs from *B. pilosa* Linn. a)5000x b)1500x magnifications

Researchers have reported that the synthesized silver nanoparticles from various plant sources are mostly spherical in shape and are of different sizes (Arumagam et al., 2014).The exterior surfaces of silver nanoparticles become shiny on the spot's spherical shape (Shameli et al., 2012). The same result was reported by Shameli et al. (2012) in which AgNPs with spherical shape was synthesized using *Curcuma longa* tuber powder.

Fig. 5 shows the elemental composition of the biosynthesized AgNPs which was carried out using electron dispersive x-ray (EDX) spectroscopy.

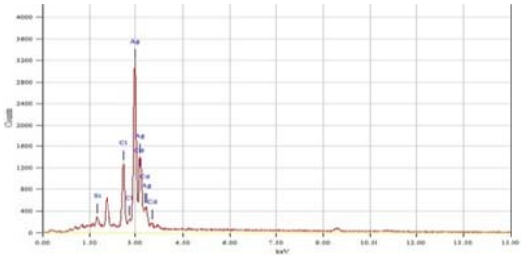

Fig. 5: EDX spectrum of the biosynthesized silver nanoparticles

Among the present elements, silver has the highest atomic percentage value equivalent to 66.23%. Presence of trace elements in the analysis is due to the characterization procedures and substrate used.

3.5. Antimicrobial screening

The antimicrobial activity of biosynthesized AgNPs produced after 0, 24 and 48 hours of reaction of the different ratios of decocted extract of *B. pilosa* Linn. to $AgNO_3$ solution was evaluated against Gram-negative bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus, Bacillus megaterium* and *Bacillus subtilis*) and fungi (*Candida albicans*, and *Aspergillus niger*).

4. Antibacterial effect

The formation of zones of inhibition is an indication of the bactericidal activity of AgNPs, where bacteria are incapable of surviving in this zone which is possibly due to the release of silver (either in the form of silver ions or silver nanoparticles) to silver nanostructures laden disks (Agnihotri, 2014). In this antibacterial assay, 25ppm of Amoxicillin was taken as the positive control while the negative control used was distilled water. Fig. 6 shows the zones of inhibition exhibited by the biosynthesized silver nanoparticles (Table 3).

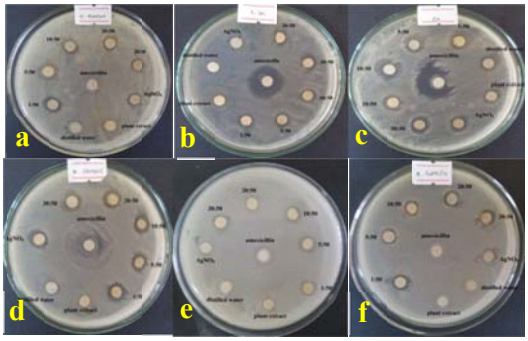

Fig. 6: Zones of inhibition against
(a) *Staphylococcus aureus* (b) *Escherichia coli*
(c) *Pseudomonas aeruginosa* (d) *Bacillus cereus*
(e) *Bacillus megaterium* (f) *Bacillus subtilis*

Table 3: Results of antibacterial activity against *Staphylococcus aureus*

Treatment	Diameter of zones of inhibition (mm)		
	0hour	24hour	48hour
1:50	4.00	4.33	3.67
5:50	4.33	3.33	2.67
10:50	4.67	3.33	3.00
20:50	5.67	3.67	2.00
30:50	6.00	4.00	4.33
Amoxicillin	3.67	4.67	4.67
5mM $AgNO_3$	4.33	1.00	1.00
Plant extract	0.00	0.00	0.00
Distilled water	0.00	0.00	0.00

Statistical analysis of the antibacterial activity of the silver nanoparticles against *S. aureus* shows that in 0 hour, the ratios 1:50, 5:50 and 10:50 are not significantly different but they are significantly different to the 20:50 and 30:50 ratios. Moreover, silver nitrate is not significantly different to 1:50, 5:50, 10:50, 20:50 and 30:50 ratios. This means that silver nitrate and the five ratios exhibit the same bactericidal effect against *S. aureus*. Thus, in 0 hour, increasing the ratio of the sample is not a factor for the effectives of the silver nanoparticles as antibacterial agent against *S. aureus*. In 24 hour, the ratios 1:50, 5:50, 10:50, 20:50 and 30:50 are not

significantly different and thus have the same efficacy against bacterial growth. These five ratios are significantly different to silver nitrate solution. The plant extract and silver nitrate are not significantly different. Thus, in 24 hour, plant extract has the ability to increase the antibacterial potential of silver nitrate solution. In 48 hour, the ratios 5:50 and 10:50 are not significantly different which means they have the same bactericidal effect. However, the ratio 5:50 is also significantly the same to the ratio 20:50 and thus they exhibit a comparable bactericidal effect. The ratio 1:50 and 30:50 are not significantly different but they are significantly different to the ratios 5:50, 10:50 and 20:50.

For the significant difference of each treatment with respect to increasing the reaction time, the ratio 5:50 in 0 hour is significantly different in 24 and 48 hour. This follows that the ratio 5:50 has the highest bactericidal efficacy in 0 hour than in 24 and 48 hour. The mean of the ratio 20:50 in 0, 24 and 48 hour are significantly different. Based on the result, the ratio 20:50 has the highest antibacterial effect in 0 hour, which is followed by 24 and then by 48 hour. The mean of the ratio 30:50 in 0 hour is significantly different to the means in 24 and 48 hour which means that the ratio 30:50 has the highest antibacterial effect in 0 hour but its bactericidal effect in 24 and 48 hour are nearly the same (Table 4).

Table 4: Results of antibacterial activity against *Escherichia coli*

Treatment	Diameter of zones of inhibition (mm)		
	0hour	24hour	48hour
1:50	6.33	2.33	3.67
5:50	5.67	3.00	2.67
10:50	5.33	3.33	3.00
20:50	6.00	3.67	2.00
30:50	6.00	3.00	4.33
Amoxicillin	4.67	5.67	5.67
5 mM $AgNO_3$	1.00	1.00	1.00
Plant extract	1.00	0.00	0.00
Distilled water	0.00	0.00	0.00

Shown in Table 5 are the results of antibacterial activity of the silver nanoparticles against *E. coli*. Statistical interpretation of the results shows that in 0 hour, there is no significant difference between the means of the sample treatments. In 24 hour, distilled water, plant extract and silver nitrate are not significantly different. Amoxicillin is significantly different to all sample treatment and has the highest antibacterial effect. The ratios 1:50, 5:50, 10:50, 20:50 and 30:50 are not significantly different which means that they have comparable antibacterial efficacy against *E. coli*. In 48 hour, the ratio 5:50 and 20:50 are not significantly different and thus they have comparable bactericidal effect against *E. coli*. The ratios 5:50 and 10:50 are not significantly different but ratio 10:50 is significantly the same to ratio 1:50. The latter ratio is not significantly different to ratio 30:50. Hence the ratios 1:50 and 30:50 have comparable antibacterial effect.

For the significant difference of each treatment with respect to increasing the reaction time, there is no significant difference in 10:50, Amoxicillin, silver nitrate, plant extract and distilled water. The means of the ratios 1:50, 5:50 and 20:50 in 0 hour are significantly different to their means in 24 and 48 hour. It follows that the three ratios exhibit the highest bactericidal effect in 0 hour than that in 24 and 48 hour.

Table 5: Results of antibacterial activity against *Pseudomonas aeruginosa*

Treatment	Diameter of zones of inhibition (mm)		
	0hour	24hour	48hour
1:50	5.00	5.00	3.33
5:50	4.00	4.33	4.00
10:50	7.00	4.33	4.00
20:50	4.33	5.67	3.33
30:50	3.67	4.33	2.00
Amoxicillin	10.33	3.33	3.33
5mM $AgNO_3$	2.67	1.00	1.00
Plant extract	0.00	0.00	0.00
Distilled water	0.00	0.00	0.00

Statistical analysis of the antibacterial activity of the synthesized silver nanoparticles against *P. aeruginosa* shows that in 0 hour, plant extract and distilled water are not significantly different but the two are significantly different to the silver nitrate solution. The standard Amoxicillin is significantly different to all sample treatments and has the highest antibacterial effect against *P. aeruginosa*. The ratios 1:50, 5:50, 20:50 and 30:50 are not significantly different. Hence, they exhibit a comparable bactericidal effect. The ratio 10:50 is significantly different to other four ratios (1:50, 5:50, 20:50 and 30:50). Furthermore, among the sample ratios, 10:50 has the highest bactericidal activity. In 24 and 48 hour, there is no significant difference between the means of the sample treatments. For the significant difference of each treatment with respect to increasing the reaction time, there is no significant difference between the means of the sample treatments (Table 6).

Table 6: Results of antibacterial activity against *Bacillus cereus*

Treatment	Diameter of zones of inhibition (mm)		
	0hour	24hour	48hour
1:50	5.33	2.00	2.33
5:50	5.67	2.00	2.33
10:50	4.67	1.00	1.00
20:50	5.00	2.67	3.00
30:50	6.00	1.67	3.00
Amoxicillin	8.67	2.67	2.67
5 mM $AgNO_3$	3.33	0.00	0.00
Plant extract	0.33	0.00	0.00
Distilled water	0.00	0.00	0.00

Table 6 shows the antibacterial activity of the biosynthesized nanoparticles against *B.* cereus.

Statistical analysis shows that in 0 hour, there is no significant difference between the means of the sample treatments. In 24 hour, the ratio 1:50, 5:50, 20:50 and Amoxicillin are not significantly different, thus they exhibit comparable antibacterial effect against *B. cereus*. The ratio 30:50 is not significantly different to 10:50. In 48 hour, the ratios 1:50, 5:50, 20:50, 30:50 and Amoxicillin are not significantly different and thus have comparable bactericidal efficacy. All sample treatments aside from distilled water have means that are not significantly different in 24 and 48 hour but are significantly different to their means in 0 hour. This means that their bactericidal activities are comparable in 24 and 48 hour and which has the highest antibacterial effect in 0 hour (Table 7).

Table 7: Results of antibacterial activity against *Bacillus megaterium*

Treatment	Diameter of zones of inhibition (mm)		
	0hour	24hour	48hour
1:50	2.00	2.67	3.00
5:50	2.33	3.00	2.00
10:50	2.00	2.67	2.33
20:50	2.33	2.33	2.00
30:50	1.33	1.67	1.00
Amoxicillin	1.33	2.33	2.00
5 mM $AgNO_3$	1.00	0.00	1.00
Plant extract	0.00	0.00	0.00
Distilled water	0.00	0.00	0.00

The zones of inhibition of the synthesized silver nanoparticles against *B. cereus* are depicted in Table 7. Statistical analysis shows that in 0 hour, the ratios 1:50, 5:50, 10:50 and 20:50 are not significantly different but they are significantly different to 30:50 ratio. The Amoxicillin, silver nitrate and 30:50 ratio are not significantly different and thus, have the same bactericidal effect against *B. megaterium*. Moreover, silver nitrate is significantly different to 1:50, 5:50, 10:50 and 20:50 ratios and based on their means, these ratios has higher antibacterial activity than that of silver nitrate. In 24 hour, there is no significant difference between the means of the sample treatments. In 48 hour, the ratios 1:50, 10:50 and 20:50 are not significantly different and thus have comparable effect against bacterial growth inhibition. Moreover, the ratios 10:50 and 20:50 are not significantly different to ratios 5:50 and 30:50 ratios. The silver nitrate solution, 5:50, 10:50 and 30:50 are not significantly different and thus, have comparable bactericidal activity.

For the significant difference of each treatment with respect to increasing the reaction time, there is no significant difference observed.

Statistical analysis of the antibacterial activity of the synthesized silver nanoparticles is shows that in 0 hour, there is no significant difference between the means of each sample treatments. In 24 hour, silver nitrate, 10:50, 30:50 and Amoxicillin are not significantly different. This means that they exhibit a comparable bactericidal effect (Table 8).

Table 8: Results of antibacterial activity against *Bacillus subtilis*

Treatment	Diameter of zones of inhibition (mm)		
	0hour	24hour	48hour
1:50	3.67	3.67	4.00
5:50	2.67	3.33	2.33
10:50	3.00	2.33	3.00
20:50	2.67	3.00	3.33
30:50	2.33	2.67	2.67
Amoxicillin	2.33	1.67	1.00
5 mM $AgNO_3$	1.67	2.00	2.00
Plant extract	0.00	0.00	0.00
Distilled water	0.00	0.00	0.00

The ratios 10:50, 20:50 and 30:50 ratios are not significantly different. However, the ratio 20:50 is not significantly different to ratios 1:50 and 5:50. In 48 hour, silver nitrate and Amoxicillin are not significantly different. The ratios 5:50, 10:50 and 30:50 are not significantly different and thus, have comparable bactericidal activity against B. subtilis. Furthermore, the ratios 10:50 and 20:50 are not significantly different to the ratio 1:50. Among the treatments, the ratio 1:50 has the highest bactericidal efficacy. For the significant difference of each treatment with respect to increasing the reaction time, there is no significant difference observed.

4.1. Antifungal effect

The efficacy of the silver nanoparticles biosynthesized from leaf extract of *Bidens pilosa* Linn. for antifungal activity was determined against *Candida albicans* and *Aspergillus niger*. For this assay, Nystatin was used as the positive control and distilled water as the negative control. The synthesized nanoparticles showed antifungal activity to both strains of fungi as shown in Fig. 7 (Table 9).

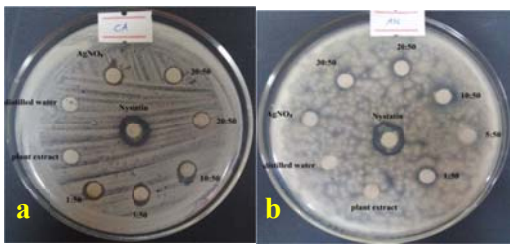

Fig. 7: Zones of inhibition against (a) *Candida albicans* (b) *Aspergillus niger*

Table 9: Results of antifungal activity against *Candida albicans*

Treatments	Diameter of zones of inhibition (mm)		
	0hour	24hour	48hour
1:50	3.00	1.33	1.67
5:50	2.33	4.00	1.67
10:50	2.67	2.67	2.00
20:50	2.00	3.33	1.00
30:50	2.00	3.67	1.33
Nystatin	1.33	8.88	7.33
5 mM $AgNO_3$	1.67	1.33	2.00
Plant extract	0.00	1.00	0.00
Distilled water	0.00	0.00	0.00

Statistical analysis of the antifungal activity of the synthesized silver nanoparticles shows that in 0 and 24 hour, there is no significant difference between the means of each sample treatments. In 48 hour, silver nitrate, 1:50, 5:50, 10:50 and 30:50 are not significantly different. This means that they exhibit comparable antifungal effect. The ratio 20:50 has also no significant difference to ratios 1:50, 5:50, and 30:50. Nystatin is significantly different to all sample treatments and thus has the highest antifungal activity. For the significant difference of each treatment with respect to increasing the reaction time, there is no significant difference observed in ratios 1:50, 10:50, 20:50 and 30:50. The means of 5:50 ratio in 0 hour and in 48 hour are not significantly different but they are significantly different in 24 hour. This means that 5:50 exhibits comparable antifungal activity in 0 and 48 hour and it has greater antifungal activity in 24 hour. Clearly, there is no pattern that would describe the trend of the effectiveness of the sample to inhibit the growth of *C. albicans* (Table 10).

Table 10: Results of antifungal activity against *Aspergillus niger*.

Treatments	Diameter of zones of inhibition (mm)		
	0hour	24hour	48hour
1:50	3.67	1.00	2.00
5:50	2.67	2.67	2.33
10:50	3.00	3.33	1.67
20:50	2.67	2.00	2.00
30:50	2.33	1.33	1.00
Nystatin	1.33	9.00	6.67
5 m$MAgNO_3$	1.67	2.00	2.00
Plant extract	0.00	0.00	0.00
Distilled water	0.00	0.00	0.00

Based on the statistical analysis of the silver nanoparticles against *A. niger*, there is no significant difference between the means of each sample treatments in 0 hour. In 24 hour, silver nitrate, 1:50, 5:50, 20:50 and 30:50 are not significantly different. This means that they exhibit comparable antifungal effect. The ratio 10:50 is significantly different to all sample ratios and has the highest antifungal activity among the sample ratios. In 48 hour, ratios 1:50, 20:50 and silver nitrate are not significantly different and thus, have comparable antifungal activity. The ratio 5:50 is not significantly different to ratios 1:50, 20:50 and silver nitrate. The ratio 10:50 is not significantly different to ratios 1:50, 20:50 and silver nitrate. This means that 5:50 and 10:50 have comparable antifungal activity with 1:50, 20:50 and silver nitrate. However, 5:50 and 10:50 have antifungal activity that is not comparable. For the significant difference of each treatment with respect to increasing the reaction time, there is no significant difference observed. Hence, increasing the reaction time of the sample ratios is not a significant factor for the effectiveness of the sample to inhibit the growth of *A. niger*.

5. Conclusion

Biosynthesis of silver nanoparticles was made possible using fresh leaf extract of *Bidens pilosa* Linn.. The nanoparticles were characterized using UV-Vis spectrophotometry, FTIR spectroscopy and SEM-EDX analysis. The biosynthesized silver nanoparticles showed antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, *B. subtilis*, *B. cereus*, *B. megaterium*, *C. albicans* and *A. niger*.

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