Rapid Biogenic and Uv-Vis Spectroscopic Study of Silver Nanoparticle Synthesis by Methanolic Extract of Piper Species

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Abstract
Nanotechnology is one of the most developing areas which involve utilization of materials with structural dimension in of 1-100 nm. This technology opened a pathway for the synthesis of silver nanoparticle by various physical and chemical methods. The biosynthesis method from plant extracts are considered as alternative as it is relatively simple, nontoxic, and environmentally friendly methods. The objective was to synthesize and characterize silver nanoparticle via ultraviolet visible spectroscopy of silver nanoparticles from three piper species which are Piper nigrum, Piper sarmentosum and Piper betel. Silver nanoparticles were prepared by the reaction of 1 mM of 90 ml silver nitrate and 10 ml methanolic leaf extract of each type of plant that act as reductant and stabilizer. The synthesized nanoparticle was characterized by color changes from green into dark brown with increasing intensity of the color from 10 minutes until 48 hour indicating the completion of silver nanoparticle formation at 48 hour. A UV–Vis spectrum of the methanolic medium containing silver nanoparticles demonstrated a peak at 413 nm, 417 nm and 420 nm for Piper nigrum, Piper sarmentosum and Piper betel respectively that corresponds to the plasmon absorbance of silver nanoparticles. The maximum percentage increase in the rate of absorbance was found specifically at 24 hour in all the three leaf. In conclusion piper species is a source for synthesizing silver nanoparticles and Piper nigrum being an excellent source for the synthesis due to the narrow, sharp peak and showing maximum percentage increase at 24 hour among all the three plants. This study exhibited the potential of three methanolic extract of piper species for the green synthesis of silver nanoparticles.

Keywords:
Piper sp, silver nanoparticle, ultraviolet visible spectroscopy, plasmon absorbance, green synthesis
Introduction

Nanotechnology is a crucial branch of contemporary research focusing with designation, formation, and manipulation of particles structure with a size range of 1-100 nm and the term Nanotechnology was invented in the year of 1974, by Professor Norio Taniguchi of Tokyo Science (Shukla, 2014). It is a rapidly developing science of synthesizing and utilizing Nano-sized particles. Nanoparticle is classified into organic nanoparticle and inorganic nanoparticle. Organic nanoparticle consists of carbon nanoparticles whereas inorganic nanoparticle consists of magnetic nanoparticles, and noble metal nanoparticles such as gold, silver and platinum (Kwaja et al., 2018).

Three modes of synthesizing nanoparticle are by chemical, physical and biological means. The methods of chemical and physical that are stated in the literature are extremely costly and there is an involvement of toxic, perilous chemicals like stabilizers which may cause environmental issues and biological risks. The use of toxic compounds forms a boundary for their application and also arising issues economically (Firdhouse et al., 2012). This has raised an alternative way of synthesizing nanoparticle which is by the green method. Silver nanoparticles currently synthesized using many natural products like, alfalfa sprouts, and chilli (Capsicum annuum), green tea (Camellia sinensis) and guava (Psidium guajava) leaf extract respectively (Saware et al., 2014). The three plants selected for the present study was selected from piper species which are Piper nigrum, Piper sarmentosum and Piper betel.

Methods

Collection and Identification of Plant

Three types of piper species leaves were collected. Fresh and green leaves of Piper nigrum were obtained from Pagar Alam, South Sumatera, Indonesia. Piper sarmentosum leaves were collected from a farm in Kota Bahru Kelantan and leaves of Piper betel were collected from a farm in River Estate, Kuala Selangor. Identification and certification of the specific plant species was sent and obtained from University Putra Malaysia (UPM) in time duration of two weeks.

Plant Handling and Preparation of Plant Powder

Leaves of piper plant samples were washed for three times with adequate amount of running tap water and rinsed with distilled water. The leaves were air dried at room temperature of 20-25°C for a week followed by oven drying at 50°C for 5 hours. The dried leaves were powdered using an electric grinder to form fine powders. The weight of piper sp. powder was measured using an analytical balance. The same procedures were repeated with all three piper nigrum and piper sarmentosum (Praba et al., 2014).

Solvent Extraction

10g of dried powder piper plant was and mixed with 100 ml of methanol. The mixture was left overnight for infusion. The mixture was heated in water bath at 50°C for 2 hours. The sample was then filtered using filter paper, and the filtrate is the piper betel extract that has been used as reducing agent in the nanoparticle synthesis. Filtration step was repeated for 3 to 4 times for a clear solution and the filtrate obtained was collected to be stored in refrigerator at 4 °C and used within one week. The same procedure was repeated with Piper nigrum and Piper sarmentosum (Gokak et al., 2014).

Preparation of Silver Nitrate Aqueous Solution (AgNO3)

An accurate mass of 0.017g of silver nitrate was weighed and dissolved with 100 ml of distilled water to prepare 1 mM of silver nitrate solution. It was stored in amber colour bottle until further use to prevent any light mediated reaction (Aruna et al., 2014).
Synthesis of Silver Nanoparticles

10 ml of methanolic extract of *Piper betel* in conical flask were slowly added with 90ml of 1 mM AgNO\(_3\) aqueous solution. The mixture was constantly stirred at 120 rpm and heat at a at 50° C. Heating and stirring was continued until the color of the solution was changed. Then it was removed from heating and stirred until cooled to room temperature. A control is maintained without addition of leaf extract in the silver nitrate solution that shows no color changes (Aruna et al., 2014).

Characterization by Using Ultraviolet Visible Spectroscopy

The complete bioreduced of silver metal ions to silver nanoparticle was analyzed via UV-visible spectrophotometer with the wavelength range of 380 nm to 700 nm. 1 ml of the sample suspension was diluted with 2 ml of distilled water and subsequent scan in UV-vis spectra. The time interval the reading observed was at 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 24 hours and 48 hours. (Banerjee et al., 2014)

Data Analysis

Qualitative technique was used in which the visible color change was observed and the and UV-Vis Spectrum was analyze base on the interest wavelength for silver nanoparticles formed.

Result

Figure 1 shows the colour changes of solution and formation of silver nanoparticles on the digital photographs. (A) was labelled as 1 mM silver nitrate solution without leaf extract, (B) referred to tube with methanolic extract of *Piper betel*, (C) 1 mM silver nitrate solution with methanolic extract of *Piper betel* leaf after 10 minutes of incubation with the formation of yellowish brown solution, (D) 3 hour incubation with the formation of dark yellowish brown solution, (E) 24 hours with the formation of light brown solution and (F) 48 hours with formation of dark brown solution.

Figure 2 shows the UV-Vis Spectrum of *Piper betel* which shows the absorbance peak to be remained constant at 420 nm starting from 10 minutes until 48 hours. The absorbance peak was observed to become sharp and broader as time increases. The absorbance value observed to increase as time increases from 10minutes to 24 hour and the least increase was observed at 48 hours.

Table 1 shows the percentage increase in absorbance of methanolic *Piper betel* extract and silver nitrate mixture at the time interval of 30 minutes, 1 hours, 2 hours, 3 hours, 4 hours, 24 hours and 48 hours. The maximum percentage increase in absorbance is observed at 24 hours with 9.12% with an absorbance value of 3.649.

Figure 3 shows the trend of colour changers on digital photographs of (A) 1 mM AgNO\(_3\) without leaf extract (B) methanolic extract of *Piper sarmentosum* leaf (C) 1 mM AgNO\(_3\) with methanolic extract of *Piper sarmentosum* leaf after 10 min of incubation with the formation of yellowish solution (D) 3 hour incubation with the formation of yellowish brown (E) after 24 hours with the formation of light brown solution and (F) after 48 hours with the formation of dark brown solution.

Figure 4 shows the graph of absorbance against wavelength (nm) of *Piper sarmentosum* which shows the absorbance peak to be remained constant at 417 nm starting from 10 minutes until 48 hours. The absorbance peak was observed to become sharper and narrower as time increases. The absorbance value was also observed to increase as time increases from 10minutes to 24 hour and a less increase was observed at 48 hours.

Table 2 shows percentage increase in absorbance of methanolic *Piper sarmentosum* extract and silver nitrate mixture at the time interval of 30 minutes,1 hour, 2 hours, 3 hours, 4 hours, 24 hours and 48 hours. The maximum percentage increase in absorbance is observed at 24 hours with 9.50% with an absorbance value of 4.793.

Figure 5 shows digital photographs of (A) 1 mM silver nitrate without leaf extract (B) *Piper nigrum* leaf extract (C) 1 mM silver nitrate with methanolic extract of *Piper nigrum* leaf after 10 min of incubation with formation of yellowish solution (D) 3 hours incubation with the formation of yellowish brown (E) after
24 hours with the formation of light brown solution and (F) after 48 hours with the formation of dark brown solution.

Figure 6 shows the graph of absorbance against wavelength (nm) for *Piper nigrum* which shows the absorbance peak to be remained constant at 413 nm starting from 10 minutes until 48 hours. The graph also shows that the peak becomes sharper and narrower as time increases. The absorbance value observed to increase as time increases from 10 minutes to 24 hour and a less increase was observed at the 48 hours.

Table 3 shows percentage increase in absorbance of methanolic *Piper nigrum* extract and silver nitrate mixture at the time interval of 30 minutes, 1 hours, 2 hours, 3 hours, 4 hours, 24 hours and 48 hours. The maximum percentage increase in absorbance is observed at 24 hours with 35.13%.

**Discussion**

Majority of the plants from Piperaceae family are widely utilized in both tropical and subtropical region of the world as traditional medicine, spices and pest controlling substance which raises the curiosity in conducting research from the species of this genus (Fazolin et al., 2011). Recently attention has been given on utilizing the plant as a source to synthesize silver nanoparticle instead of chemical and physical method. Three types of piper species extract that were used to synthesis silver nanoparticles in this experiment are *Piper nigrum, Piper sarmentosum* and *Piper betel*.

From the research conducted the capacity of *Piper betel* to synthesize silver nanoparticle has been successfully established. The primary confirmatory of the synthesis is a visible color change was observed at 10 minutes following the addition of methanolic piper species extract to 1Mm silver nitrate solution in the ratio of 1:9. From figure 1 at 10 minutes of reaction a yellowish brown solution was observed, at 3 hour a dark yellowish brown solution was formed, at 24 hours light brown solution was formed. As time increases an increase in the intensity of the solution was observed due to the increase in the number of silver nanoparticle synthesized (Ray et al., 2009). At 48 hours of incubation of the solution a dark brown solution was formed and there was no further change in the color of the solution after this timing indicating the completion of the reaction. The change in the color of the solution is the first stage of confirmatory of the reduction of silver ion (Ag⁺) into silver nanoparticle (Ag⁰) (Phong et al., 2009). Silver nitrate solution without plant extract and the methanolic extract of piper betel was set as a control in this experiment and it does not reveal a visible change of color (Roy et al., 2012). The synthesized silver nanoparticle and its stability were characterized by UV-visible spectroscopy, as this technique has proved to be very useful for the nanoparticle analysis. In the UV-vis absorption spectrum, a strong, single peak located between the range of 400-500 nm were observed for the silver nanoparticles which provide a convenient signature for the formation of silver nanoparticle (Yakout et al., 2015).

Therefore, from figure 2 single-band absorption with peak maximum at 420 nm for *Piper betel* extract confirms the presence of silver nanoparticle. The peak was observed due to surface plasmon resonance (SPR). Surface plasmon resonance (SPR) is the excitation of electron at metal surfaces when an incident light strikes which normally occur in the surface of gold and silver. Theoretically, metal nanoparticles are surrounded by free electrons which give the absorption band due to combined vibration of electron of metal nanoparticle in resonance with light wave. The graph obtained from UV-vis of *piper betel* indicate the possibilities of forming polydispersed large nanoparticle due to a slow reduction rate by showing a show a broadening of the peak (Praba et al., 2014). The absorption spectra of nanoparticles of piper betel steadily increased in intensity as a function of time of reaction without any shift in the peak from 10 minutes to 48 hours. The reading attained a constant absorption value indicating completion of the reaction and stability of the nanoparticle (Jena et al., 2014). From table 1 the highest percentage increase in absorbance was found at 24 hours with 9.12% which confirms 24 hours as the optimum incubation time. The percentage increase in absorbance was 0.22% the least at 48 hours indicating completion of reduction of silver ions in the reaction mixture showing the unsuitability 48 hours as the optimum incubation time (Amin et al., 2012).

*Piper sarmentosum* exhibit the capability of the plant to synthesis silver nanoparticle by the formation of brown color. From figure 3 a yellowish solution was formed at 10 minutes, at 3 hours a
yellowish-brown solution was formed, at 24 hours with the formation of light brown solution and at 48 hours with formation of dark brown solution. The final coloration of dark brown confirms that silver nanoparticles exhibit dark brown color in water and this particular color is a result of surface Plasmon vibration of metal nanoparticles (Praba et al., 2015). The maximum absorption peak formed at 417 nm in Figure 4 confirming the presence of silver nanoparticle. The constant single absorption peak from 10 minutes to 48 hours proves the high stability of silver nanoparticle. UV-vis spectra show sharp narrow peak indicates the continuous formation of disaggregated nanoparticles (Amin et al., 2012). From table 2 the highest percentage increase in absorbance was found at 24 hours with 9.50% confirms 24 hours as the optimum incubation time. There is no rapid increase of intensity as recorded after 24 hours of incubation confirming the ending of bioreduction process. The percentage increase in absorbance was 0.12% the least due to the completion of reaction and showing the unsuitability of the 48 hour as the optimum incubation time (Mohammed, 2015).

Among all the three Piper species Piper nigrum is a better source for the synthesis of silver nanoparticle. From Figure 5, this plant has the highest intensity in color compared to other two plants. According to Ray in the year 2009 the higher intensity of color is an indication that there is a higher conversion of silver ion (Ag⁺) into silver nanoparticle (Ag⁰). The maximum absorption spectrum of piper nigrum shows in Figure 5 was 413 nm and a progressive increase in the characteristic peak with increase in reaction time is a clear indication of nanoparticles formation (Shekhawat et al., 2012). Further no precipitation was observed during the reaction and up to two weeks after the experiment suggesting stability of the synthesized silver nanoparticle (Roy et al., 2012). UV-vis spectra show sharp narrow peak also indicates the formation of disaggregated nanoparticles (Paulkumar et al., 2014). This single and strong band indicates that the particles are isotropic in shape and uniform size. The absorption peak intensity increased rapidly with increase in reaction time from 10 minutes to 24 hours due to the continuous formation of silver nanoparticle in the reaction system. It was, therefore, observed that an optimum time is required for the completion of reaction due to the instability in the formation of silver nanoparticles. The optimum time required for the completion of reaction was recorded to be 24 hours as all the plant shows the maximum percentage increase in absorbance at this time. Among all the three plants Piper nigrum showed the sharpest peak with the highest maximum percentage increase at 24 hours with 35.13%. (Figure 6) Thus for the ease of experimentation and to ensure the process of green synthesis of silver nanoparticle time saving the optimum incubation time is chosen as 24 hours. The lowest percentage increase was observed from 24 to 48 hours indicating that the formation of silver nanoparticle is towards the completion period (Amin et al., 2012). According to Paulkumar in 2014 a lower wavelength value confirms the synthesis of small sized nanoparticle with a higher rate of reduction of silver ion into nanoparticle. Whereas higher absorption wavelength was recorded as a result of the synthesis of large sized nanoparticle. From the result obtained Piper nigrum record the lowest wavelength which is again proving as the best choice for the synthesis of silver nanoparticle due to the small sized nanoparticle. There was no absorption peak observed in silver nitrate and plant extract showing the reduction process has not begun (Shams et al., 2014).

Conclusion

In conclusion, this study has showed the methanolic extract of piper species and silver nitrate solution has the capacity to synthesize stabilized silver nanoparticle and can be used as a source of synthesizing silver nanoparticle. Primary confirmatory for the silver nanoparticles formation was colour change into dark brown followed by UV-Vis absorption spectra of silver nanoparticles formed at 413 nm, 417 nm and 420 nm for Piper nigrum, Piper sarmentosum and Piper betel respectively. The development of nanoparticles was monitored by UV-Vis spectroscopy and its results showed that 24 hours is the optimum time that shows the maximum percentage increase in absorbance with Piper nigrum as the best source of synthesizing. Piper nigrum proven to be an excellent plant source for silver nanoparticle synthesis due to the highest percentage increase in absorbance. This study exhibited the potential of plant extract for the green synthesis of silver nanoparticles with several advantages such as low cost, easily availability of plants,
compatible that makes it suitable for medical and pharmaceutical applications as well as potential for large scale production because of commercial availability of plant. This study is considered as an attempt to save the environmental pollution by choosing the green method to synthesize silver nanoparticle.

**Acknowledgement**

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Tables and Figures

Figure 1: Colour changes of the reaction mixture using *Piper betel* at time interval of 10 minutes, 3 hours, 24 hours and 48 hours.
Figure 2: UV-Vis absorption spectrum of silver nanoparticles (SNP) from methanolic *Piper betel* leaf extract

**Table 1: Percentage increase in absorbance of *Piper betel***

<table>
<thead>
<tr>
<th>Time</th>
<th>Absorbance at 420nm of piper betel</th>
<th>%increase in absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes</td>
<td>2.723</td>
<td>5.58%</td>
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<tr>
<td>1 hour</td>
<td>3.111</td>
<td>8.18%</td>
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<td>2 hour</td>
<td>3.241</td>
<td>4.18%</td>
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<td>3 hour</td>
<td>3.278</td>
<td>1.14%</td>
</tr>
<tr>
<td>4 hour</td>
<td>3.344</td>
<td>2.01%</td>
</tr>
<tr>
<td>24 hour</td>
<td>3.649</td>
<td>9.12%</td>
</tr>
<tr>
<td>48 hour</td>
<td>3.657</td>
<td>0.22%</td>
</tr>
</tbody>
</table>
Figure 3: Colour change of the reaction mixture using *Piper sarmentosum* at time interval of 10 minutes, 3 hours, 24 hours and 48 hours.

\[
\text{AgNO}_3 + \text{p.sarmentosum Leaf extract} \quad 10\text{min}
\]

\[
\text{AgNO}_3 + \text{p.sarmentosum Leaf extract} \quad 24\text{H}
\]

\[
\text{AgNO}_3 + \text{p.sarmentosum Leaf extract} \quad 48\text{H}
\]
Figure 4: UV-Vis absorption spectrum of silver nanoparticles (SNP) from methanolic extract of *Piper sarmentosum* leaf

Table 2: Percentage increase in absorbance of *Piper sarmentosum*

<table>
<thead>
<tr>
<th>Time</th>
<th>Absorbance at 417nm of <em>Piper sarmentosum</em></th>
<th>% increase in absorbance</th>
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<tbody>
<tr>
<td>30 minutes</td>
<td>3.822</td>
<td>5.14%</td>
</tr>
<tr>
<td>1 hour</td>
<td>3.879</td>
<td>1.49%</td>
</tr>
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<td>2 hour</td>
<td>3.964</td>
<td>2.19%</td>
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<tr>
<td>3 hour</td>
<td>4.293</td>
<td>8.30%</td>
</tr>
<tr>
<td>4 hour</td>
<td>4.377</td>
<td>1.96%</td>
</tr>
<tr>
<td>24 hour</td>
<td>4.793</td>
<td>9.50%</td>
</tr>
<tr>
<td>48 hour</td>
<td>4.821</td>
<td>0.12%</td>
</tr>
</tbody>
</table>
Figure 5: Colour change in the reaction mixture using methanolic *Piper nigrum* extract time interval of 10 minutes, 3 hours, 24 hours and 48 hours.
Table 3: Percentage increase in absorbance of *Piper nigrum*

<table>
<thead>
<tr>
<th>Time</th>
<th>Absorbance at 417nm of <em>Piper nigrum</em></th>
<th>% increase in absorbance</th>
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<tr>
<td>30 minutes</td>
<td>2.975</td>
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<td>3.121</td>
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<td>2 hour</td>
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<td>4 hour</td>
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<td>48 hour</td>
<td>4.892</td>
<td>0.14%</td>
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References


