Synthesize, characterization, and anti-Parkinson activity of silver-Indonesian velvet beans (*Mucuna pruriens*) seed extract nanoparticles (AgMPn)

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**Abstract.** Parkinson is one of the progressive neurodegenerative diseases. Various efforts are made in handling this disease, one of them is the utilization of plant extracts that have anti-Parkinson activity, for example, velvet bean (*Mucuna pruriens* L.). Changing the particle size of the extract into nanoscale particle is expected to increase its anti-parkinson activity. The research was conducted to synthesize silver-velvet bean (*Mucuna pruriens* L.) seed extract nanoparticles (AgMPn) and to evaluate its antiparkinson activity through the catalepsy test in mice. The research consisted of several stages i.e. extraction of velvet bean seed powder, synthesis and characterization of AgMPn, and catalepsy test of AgMPn. Velvet bean seed powder was extracted by maceration method using ethanol-water (1:1) at pH 3 adjusted with citric acid. AgMPn was synthesized by reacting the silver nitrate (AgNO₃) solution with the extract of velvet bean seed for 40 min, dispersibility of solution during the reaction was controlled by using sonication and ultrasonic processor homogenizer. Characterization of AgMPn was done by using Fourier transform infrared (FT-IR), scanning electron microscopy-energy dispersive X-ray (SEM-EDX), and transmission electron microscopy (TEM). Catalepsy test was conducted on AgMPn at the doses of 5, 10, 15, 20 and 25 mg/kg body weight. The results of SEM-EDX and TEM showed that AgMPn formed aggregates with several shapes such as rectangle, oval, and spherical, with the average particle diameter was 36.5 nm. FT-IR spectra showed a band at 464.8 cm⁻¹ absorbance area which is typical band indicated the interaction of Ag-O of AgMPn. Catalepsy test demonstrated that AgMPn at the doses of 5, 15, and 20 mg/kg body weight lowered the catalepsy symptoms in mice significantly, with the best dose was 5 mg/kg body weight.

1. **Introduction**

Parkinson is a neurodegenerative disease and the second most common after Alzheimer. This is allegedly caused by the damage of nerve cells that implicated to the decrease of dopamine in the brain, which diminished the ability to regulate the movements, bodies, and emotions [1]. A condition characterized by inactivity, decreased responsiveness to stimuli, a tendency to maintain an immobile posture and decreased sensitivity to pain, is commonly emerge in people with Parkinson, called catalepsy [2,3]. The synthetic drugs, such as carbidopa, levodopa, and benserazide are usually used to lower this symptom. However, these drugs have serious side effects, especially in long-term use.
Over the years, people considered finding an alternative medicine which has lower side effects and lower cost, such as the utilization of the plant extracts. *M. pruriens* has been reported as a potential plant to lower catalepsy due to the content of L-dopa in the seed [4-7]. L-dopa is a precursor of dopamine, able to cross the blood-brain barrier, hence it can help to improve the lack of dopamine [8]. *M. pruriens* seed from Indonesia has been reported to contain L-dopa as much 7.56 to 13.9%, in addition to other contents such as alkaloids, steroids, saponins, and tannins [9]. The seed showed a promising anti-parkinson activity [10-12]. The seed extracts at dose of 200 mg/kg and 400 mg/kg body weight could lower the catalepsy significantly in mice (*Mus musculus*) [5].

Nanoparticles ameliorate the compatibility and bioavailability of the extract, hence it improves the performance of the drugs in the treatment of several diseases, including Parkinson. Since nanoparticles enhanced advantages over the larger particles due to their size, distribution, and morphology, synthesis of nanoparticles has been a great demand. Among the metallic nanoparticles, silver nanoparticles are many selected to develop in the treatment of diseases [13]. It due to their good stability, conductivity, catalytic properties, and bioactivity [14]. A method to synthesize nanoparticles involving biological components such as microorganisms and plant extracts as reducing agents is more environmentally friendly because it does not use the toxic chemicals, can be used in large-scale production, and is energy saving. Synthesis nanoparticles mediated by plant extract is faster, easier and cheaper compared to it mediated by microorganism [15-17].

The previous study reported that the nanoparticle from *M. pruriens* seed extract could improve motoric disorders in animals induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) significantly compared to *M. pruriens* seed extract itself [18]. This finding showed that the nanoparticle extract provides a better bioactivity. Unfortunately, there was no scientific study on the biosynthesis of silver nanoparticles- *M. pruriens* seed extract (AgMPn) from Indonesia. In this paper, we report the biosynthesis, characterization, and catalepsy test of AgMPn from Indonesia as a candidate of nanoparticle herbal medicine in the treatment of Parkinson disease.

2. Methods

2.1. Materials
*M. pruriens* seeds were obtained from Bantul, Yogyakarta, Indonesia. The chemical used included: ethanol (96%), citric acid p.a, AgNO₃ p.a, distilled water, haloperidol, PGA (Pulvis Gummi Arabicum) 1% and animal feed (CP551).

2.2. Extraction of *M. pruriens* seeds
The seeds were sun-dried and ground into a powder, then macerated with ethanol-water (1:1) for 3×24 h with the addition of citric acid up to pH 3. The extract (12 Liters) was evaporated at 40 °C under low pressure in a rotary vacuum evaporator then dried using a freeze dryer to obtain the dry extract. It was obtained 242.70 gram (4.11%) of the dry extract from 5.9 kg of the seed powder.

2.3. Synthesis of AgMPn
*M. pruriens* seed extract solution was prepared by dissolving 5 gram of the seed in 100 mL of distilled water then stirred for 15 min and filtered to give 50,000 ppm of extract solution. AgMPn was prepared by adding the extract solution into 0.0589 M AgNO₃ solution dropwise with a volume ratio of 1:1. The mixture was stirred for 20 min with sonication and 20 min with an ultrasonic homogenizer and kept at room temperature for 24 h. The suspension was centrifuged at 8,000 rpm, washed several times using distilled water, and dried at room temperature to produce AgMPn powder.

2.4. Characterization of AgMPn
Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX). Scanning electron microscopy (SEM) and elemental analysis (energy dispersive X-ray analysis: EDX) were carried out using Hitachi SU3500 with coating ion sputter Hitachi MC1000. It was conducted to determine the morphology, size
and elemental composition of AgMPn. Samples were crushed, coated with Au and placed on the sample container then analyzed. 

*Transmission Electron Microscopy* (TEM). Characterization using TEM conducted to determine the shape and size of the AgMPn. Samples were mixed with a dispersing agent, placed on the sample grid then analyzed.

*Fourier Transform Infrared* (FT-IR). Characterization of functional groups contained in the extract and AgMPn was done by FT-IR spectroscopy Shimadzu 8400. Samples were prepared in KBr pellets. The measurement was conducted in a wave number range 4000-400 cm⁻¹.

2.5. *Catalepsy Test*

2.5.1. *Preparation of Animals.* 3-months-old healthy male mice with 18-35 body weight were employed in catalepsy test. Animals were housed in polypropylene cages and acclimated for a week prior to the experimentation period under standard conditions (± 22 °C). A standard diet (CP551) and water were provided to animals.

2.5.2. *Experimental Design.* The mice were randomly distributed to nine groups (Table 1) with three mice each. Observations were done 30 min after administration of haloperidol suspension. Haloperidol was given to mice orally 30 min after oral administration of the vehicle (PGA 1%) or L-dopa, or the extract, or AgMPn at the respective dose. The intensity of catalepsy was measured as the length of time the mice hang on at 50 cm in height with both forelegs holding the horizontal wire with 0.5 cm in diameter (Fig. 1). Mice were catalepsy if hang on the wire for more than 15 sec.

**Table 1. Group distribution for catalepsy test**

| No | Group                | Treatment                        |
|----|----------------------|----------------------------------|
| 1  | Normal               | water                            |
| 2  | negative control     | vehicle (PGA 1%)                 |
| 3  | positive control     | L-dopa 10 mg/kg body weight      |
| 4  | the extract          | *M. pruriens* seed extract 200 mg/kg body weight |
| 5  | dose I               | AgMPn 5 mg/kg body weight        |
| 6  | dose II              | AgMPn 10 mg/kg body weight       |
| 7  | dose III             | AgMPn 15 mg/kg body weight       |
| 8  | dose IV              | AgMPn 20 mg/kg berbadan          |
| 9  | dose V               | AgMPn 25 mg/kg berbadan          |

**Figure 1.** Design of catalepsy test

2.5.3. *Data analysis.* The data of catalepsy test were analyzed statistically using one-way analysis of variance (ANOVA) followed by Dunnett’s test to evaluate significant differences between the control
and the treated groups. All statistical analysis was performed using SPSS 22.0 software. P < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Synthesis and Characterization of AgMPn
Qualitatively, the formation of AgMPn indicated by the colour change of the AgNO3 solution from colourless to yellow solution and intense brown after 40 min mixed with the extracts. The colour change of the solution occurs during the sonication process. The result of characterization using SEM showed that AgMPn formed aggregates with uneven surface and varying sizes between 53-117 nm. Based on TEM images, AgMPn formed aggregates with several shapes, mostly rectangular, in spite of other shapes such as oval and spherical, with the average particle diameter was 36.5 nm.

![Figure 2. SEM image of AgMPn](image1)

![Figure 3. TEM image of AgMPn](image2)

![Figure 4. EDX spectrum of AgMPn](image3)

The EDX spectrum showed the elemental composition contained in AgMPn, where silver (57.48%) was the greatest element compared to carbon (18.47%), nitrogen (6.56%), oxygen (17.2%), and chloride (0.29%). The EDX spectra also exhibited the strong signals for silver and while the signal for carbon, oxygen, nitrogen, and chloride was weak which may be derived from biomolecules contained in velvet bean extract that attached to the surface of silver nanoparticles. This result indicated the reduction of silver ions into silver metal.

The functional groups contained in the extracts and AgMPn was confirmed by FT-IR analysis. This characterization was also conducted to determine the biomolecules contained in the extracts involved in the formation of AgMPn. Based on FT-IR spectra of the extract (Fig. 5), there were absorbance bands at area 3384.8; 1627.8; 1529.4; 1400.2; 1288.4; 1118.6; and 1074.3 cm⁻¹. An intense and broad absorption band at 3384.8 cm⁻¹ assigned to the overlapping of O-H stretching vibration of flavonoids, alkaloids, polyphenols, alcohols or water and N-H stretching vibration of amine compounds, due to hydrogen bonding. The absorption band at 1627.8 cm⁻¹ referred to C=C stretching vibration. The absorption band in 1529.4 cm⁻¹ referred to N-H bending vibration of amine which is possible to be derived from the L-DOPA, while an absorption band intense enough at absorbance area of 1400.2 cm⁻¹
referred to C-H bending vibration of \( sp^2 \) carbon, which both were possible to be derived from the aromatic ring of amino acid. The weaker absorption band at 1288.4 cm\(^{-1}\) corresponded to \( =C-O \) stretching vibration of aromatic compounds. The absorption band at 1074.3-1118.6 cm\(^{-1}\) referred to C-O stretching vibration of amino acid.

In the FT-IR spectrum of AgMPn (Fig. 6), there are several changes in the intensity of the absorbance and shifts in absorbance area. This finding indicated the presence of interactions of the compounds in the extracts with the metal to form AgMPn. The presence of N-H, O-H, C=C, and C-O functional groups referred to the presence of amino acid or L-dopa that possible involved in the synthesis of AgMPn as reducing agents. The hydroxyl groups contained in L-dopa could act as a reducing agent, which oxidized to form dopaquinone, indicated by the decrease in the intensity of the absorbance. The absorbance band at 464.8 cm\(^{-1}\) indicated the interaction of Ag-O in AgMPn.

| Table 2. The wavelength numbers in the FT-IR spectra of M. pruriens seed extracts and AgMPn |
|-------------------------------------|---|---|
| Functional group | Wavelength (cm\(^{-1}\)) | | |
| O-H (stretching), N-H (stretching) | 3384.8 | 3222.8 |
### Functional group | Wavelength (cm⁻¹)
--- | --- | ---
C-H (stretching) | - | 2922.0
C=C (stretching) | 1627.8 | 1627.8
N-H (bending) | 1529.4 | 1512.1
C-H sp² bending | 1400.2 | 1440.7
C-O aromatic | 1288.4 | 1382.9
C-O (stretching) | 1074.3-1118.6 | 1062.7
Ag-O | - | 464.8

3.2. Catalepsy Test

The catalepsy observation was performed 60 min after the administration of haloperidol and L-DOPA or the extract or AgMPn orally. The intensity of the catalepsy was measured as the time of the mice hang on a 15 cm height rod with both front legs hold a wire with 0.5 cm in diameter. Mice were catalepsy if they hang on the wire for more than 15 sec. The data of the catalepsy test are shown in Fig. 7. Based on Fig. 7, the negative control group had a hanging time of more than 20 sec, means the mice were catalepsy. Group treated by the extract and AgMPn on the all five doses had an average of hanging time of fewer than 20 sec, means the mice were not catalepsy. However, the hanging time was longer than the normal and positive control groups. It indicated that the intensity of catalepsy in mice treated with the extract and the AgMPn decreased, but its decrease was not same compared to the administration of L-DOPA. Based on Fig. 7, AgMPn at doses of 5, 10, 15, 20 and 25 mg/kg body weight was able to lower catalepsy. The groups treated with AgMPn at doses of 5, 10, 15 had shorter hanging time compared to the group treated with the extract, indicated that AgMPn at doses of 5, 10, 15 was able to lower the catalepsy better than the extract.

![Figure 7. Diagram of catalepsy test](image-url)

A statistical test using one-way ANOVA followed by Dunnett post hoc test was conducted to determine the significance of AgMPn in lowering catalepsy in mice. The limit of significance in this study was 0.05 with a 95% confidence level. If the P value less than 0.05, means that the data is significantly different. AgMPn has a significant effect in lowering catalepsy if it has P less than 0.05 compared to negative control. The results of the statistical test are shown in Table 3.

| Group | Hanging time (sec) |
| --- | --- |
| Normal | 3.67 |
| Negative control | 22.67 |
| Positive control | 11.00 |
| The extracts | 5.33 |
| AgMPn 5 mg/kg | 6.00 |
| AgMPn 10 mg/kg | 8.00 |
| AgMPn 15 mg/kg | 12.33 |
| AgMPn 20 mg/kg | 12.67 |
| AgMPn 25 mg/kg | 0 |

**Figure 7. Diagram of catalepsy test**
a dose of 5, 10, and 15 mg/kg body weight lowered catalepsy until the mice were normal (healthy) and the activity of AgMPn at a dose of 5, 10, and 15 mg/kg body weight to lower catalepsy was equal to its L-DOPA. In the other hand, although the administration of AgMPn at a dose of 5, 10, and 15 mg/kg body weight to lower the catalepsy was better than the extract, statistically its activity in lowering catalepsy was not significantly different.

### Table 3. The results of statistical test for AgMPn groups

| Group               | AgMPn 5 mg/kg | AgMPn 10 mg/kg | AgMPn 15 mg/kg | AgMPn 20 mg/kg | AgMPn 25 mg/kg |
|---------------------|---------------|---------------|---------------|---------------|---------------|
| Normal              | 0.990         | 0.961         | 0.711         | 0.164         | 0.143         |
| Negative control    | 0.004*        | 0.005*        | 0.012*        | 0.083         | 0.096         |
| Positive control    | 0.990         | 0.961         | 0.711         | 0.164         | 0.143         |
| The extract         | 0.532         | 0.636         | 0.915         | 0.997         | 0.992         |

*P<0.05 is considered as significantly different. Statistical test was conducted by one-way ANOVA followed by Dunnett post hoc test.

### 4. Conclusion

Silver-velvet bean (*Mucuna pruriens* L.) seed extract nanoparticles (AgMPn) has been successfully synthesized by reacting the silver nitrate (AgNO₃) solution with the extract of velvet bean seed for 40 min. Dispersibility of solution during the reaction was controlled by using sonication and ultrasonic processor homogenizer. The AgMPn obtained has a particle size of 36.5 nm and formed aggregates with several shapes such as rectangle, oval, and spherical, confirmed by SEM-EDX and TEM. FT-IR spectrum of AgMPn showed a band at 464.8 cm⁻¹ absorbance area which is typical band indicated the interaction of Ag-O of AgMPn. Catalepsy test demonstrated that AgMPn at the doses of 5, 10, and 15 mg/kg body weight lowered the catalepsy symptoms in mice significantly, with the best dose was 5 mg/kg body weight.

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