Synthesize of zinc nanoparticles using Indonesian velvet bean (Mucuna pruriens) extract and evaluate its potency in lowering catalepsy in mice

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Abstract. This study aims to synthesize zinc nanoparticles using Indonesian velvet bean (Mucuna pruriens) seed extract and evaluate its potency in lowering catalepsy in mice. The research conducted consist of extraction of M. pruriens seed powder, synthesis of zinc-M. pruriens seed extract nanoparticles (Zn-MPn), characterization of Zn-MPn, and catalepsy test of Zn-MPn. M. pruriens seed powder was extracted by maceration using ethanol-water (1:1) at pH 3 adjusted with citric acid. The Zn-MPn was synthesized by reacting zinc acetate dihydrate (Zn(CH3COO)2.2H2O) solution with M. pruriens seed extract for 40 min, dispersibility of the reaction was controlled by using sonication and ultrasonic homogenizer. The Zn-MPn obtained was characterized by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX), transmission electron microscopy (TEM), and Fourier-transform infrared (FTIR). Catalepsy test of Zn-MPn was conducted at doses of 5, 10, 15, 20 and 25 mg/kg body weight. The results of SEM-EDX and TEM analysis showed that the Zn-MPn formed nanoparticles with a particle diameter of 55 nm. Based on FTIR analysis, the absorption band at 464.8 cm−1 was a typical absorption indicated the Zn-O interaction on Zn-MPn. Catalepsy test showed that Zn-MPn on the all five doses were able to lower the catalepsy in mice with the best dose was 10 mg/kg body weight.

Keywords: Mucuna pruriens, velvet bean, nanoparticle, zinc, antiparkinson, catalepsy

1. Introduction
Parkinson is a neurodegenerative disease and the second most common after Alzheimer. Parkinson disease is allegedly caused by the damage of nerve cells that causes the decrease of dopamine in the brain and leads a person to have less ability to regulate the movements, bodies, and emotions [1]. One of the symptoms in people with Parkinson is catalepsy, a disorder of the central nervous system, characterized by muscle rigidity, immobile posture and decreased sensitivity to pain [2], [3]. The efforts to lower catalepsy are still dominated by using synthetic drugs, such as carbidopa, levodopa, and benserazide. However, these drugs have serious side effects, especially in long-term use [4].
Traditional medicine using plant extracts has been chosen as an alternative due to the lower side effects. *M. pruriens* or velvet bean is considered as a potential plant to lower catalepsy [5], [6]. *M. pruriens* seed contains L-dopa, a precursor of dopamine that is able to cross the blood-brain barrier and help to improve the lack of dopamine [7]–[9]. *M. pruriens* seed from Indonesia has been reported to contain alkaloids, steroids, saponins, tannins and 7.56 to 13.9% of L-dopa [10]. Various studies have reported the activity of *M. pruriens* seed as a promising antiparkinson [11]–[13]. Antiparkinson pharmacological studies of *M. pruriens* seed extract on mice (*Mus musculus*) showed that the extract at doses of 200 mg/kg and 400 mg/kg body weight could lower the catalepsy significantly [6].

The changing of the extract into a nanoparticle is claimed to have advantages. Nanoparticles ameliorate the compatibility and bioavailability of the extract, hence it improve the performance of the extract as drugs in the treatment of several diseases, including Parkinson. The ability of the nanoparticle drugs to reach the target directly makes the drugs are not widely distributed in the body hence it impacts to the low the side effects of the drugs. Nanoparticle drugs are also easily suspended in a liquid and capable to cross the organs and tissues [14]. 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, zinc nanoparticles are very interesting to explore because of their physical and chemical properties. Zinc nanoparticles have low-toxicity, high biocompatibility, and are biodegradable that make them widely applied in the biomedical field. Some of them have been used in a number of drug formulations due to their antibacterial, antifungal, and disinfecting properties [15], [16].

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 [17]–[19]. *M. pruriens* seed extract has been reported to be used in the nanoparticle synthesis, and the nanoparticle obtained has been proved 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 [20]. This result suggests that nanoparticle provides a better bioactivity.

The empirical evidence shows the potency of *M. pruriens* in the development of a promising antiparkinson medicine. Unfortunately, there is no scientific study on the biosynthesis of zinc nanoparticles—*M. pruriens* seed extract (Zn-MPn) from Indonesia. In this paper, we report the biosynthesis and characterization of Zn-MPn from Indonesia as a candidate of nanoparticle herbal medicine in lowering catalepsy as one of the symptoms of Parkinson disease.

2. Materials and Methods

2.1. Material

*M. pruriens* seeds were obtained from Bantul, Yogyakarta, Indonesia. The chemical used included: ethanol (96%), citric acid p.a., (Zn(CH$_3$COO)$_2$)$_2$H$_2$O) p.a, distilled water, haloperidol, PGA (Pulvis Gummi Arubicum) 1% and animal feed (P551).

2.2. Extraction of *M. pruriens* seeds

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

2.3. Synthesis of Zn-MPn

5 gram of *M. pruriens* seed extract was dissolved in 100 mL of distilled water then stirred for 15 min and filtered to give 50,000 ppm of extract solution. Zn-MPn was prepared by adding the extract
solution into 0.04 M (Zn(CH$_3$COO)$_2$.2H$_2$O) 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 5,000 rpm and washed several times using ethanol, then dried in an oven at a temperature of 40 °C to produce a Zn-MPs powder.

2.4. Characterization of Zn-MPs

2.4.1. 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 Zn-MPs. Samples were crushed, coated with Au and placed on the sample container then analyzed.

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

2.4.3. Fourier Transform Infrared (FT-IR). Characterization of functional groups contained in the extract and AgMps 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 Zn-MPs 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 seconds [34, 35].

| No | Group           | Treatment                                      |
|----|-----------------|-----------------------------------------------|
| 1  | normal          | water                                         |
| 2  | negative control| haloperidol 5 mg/kg body weight               |
| 3  | positive control | L-DOPA 5 mg/kg body weight                    |
| 4  | the extract     | M. pruriens seed extract at 200 mg/kg body weight |
| 5  | dose I          | Zn-MPs at 5 mg/kg body weight                 |
| 6  | dose II         | Zn-MPs at 10 mg/kg body weight                |
| 7  | dose III        | Zn-MPs at 15 mg/kg body weight                |
| 8  | dose IV         | Zn-MPs at 20 mg/kg body weight                |
| 9  | dose V          | Zn-MPs at 25 mg/kg body weight                |
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 Zn-MPn
The colour of zinc acetate dihydrate (Zn(CH$_3$COO)$_2$.2H$_2$O) solution changed from colourless into black when the extract solution was added. The colour change of the solution occurs during the sonication process. It indicated the formation of Zn-MPn qualitatively. Based on SEM and TEM images, Zn-MPn has a particle size of 55 nm with an oval in shape. The EDX spectrum showed the elemental composition contained in Zn-MPn, where the composition of zinc was 18.3% and the others were carbon (38.5%) and oxygen (26%). The composition of carbon and oxygen may be derived from biomolecules contained in M. pruriens seed extract that contributed to the formation of zinc nanoparticles. This result indicated the reduction of zinc ions into zinc metal.
Characterization with FTIR was performed on both *M. pruriens* seed extract and Zn-MPn. It aims to determine the biomolecules contained in *M. pruriens* seed extract involved in the formation of Zn-MPn. Based on FT-IR spectra of the extract (Fig. 5), an intense and broad absorption band at 3384.8 cm\(^{-1}\) indicated the overlapping of O-H stretching vibration of flavonoids, alkaloids, polyphenols, alcohols or water with N-H stretching vibration of amine compounds due to hydrogen bonding. The absorption band at 1627.8 cm\(^{-1}\) referred to N-H bending vibration of amine which is possible to be derived from the L-DOPA, while the absorption band at 1529.4 cm\(^{-1}\) referred to C=C stretching vibration. The absorption band at 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 absorption band in absorption area of 1288.4 cm\(^{-1}\) indicated the C-O stretching vibration of aromatic compounds and the absorption band at 1074.3-1118.6 referred to C-O stretching vibration of amino acid.

![Figure 5. Spectrum of the extract (black line) and Zn-MPn (green line)](image)

In the FTIR spectrum of Zn-MPn, there are several changes in the intensity of the absorbance and shifts in absorbance area. There was a new absorption band in absorption area of 464.8 cm\(^{-1}\) which is a typical band for the Zn-O interaction. This finding indicated the presence of interactions of the compounds in the extracts with the zinc metal to form Zn-MPn. 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 Zn-MPn 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.
Table 2. The wavelength numbers in the FTIR spectra of *Mucuna pruriens* seed extract and Zn-MnPn

| Functional group                  | Wavelength (cm⁻¹) |
|----------------------------------|-------------------|
|                                  | *M. pruriens* seed extract | Zn-MnPn       |
| O-H (stretching),                | 3384.8            | 3315.4        |
| N-H (stretching)                 |                   |                |
| C-H (stretching)                 | 2960.5-2931.6     | 2960.5-2931.6 |
| N-H (bending) (NH₃)              | 1627.8            | 1639.4        |
| N-H                               |                   |                |
| C≡C (stretching)                 | 1529.4            | 1531.4-1542.9 |
| C-H (bending)                    | 1400.2            | 1440.7-1398.3 |
| C-O aromatic                     | 1288.4            | 1286.4-1240.1 |
| C-O (stretching)                 | 1074.3-1118.6     | 1072.3        |
| Zn-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 Zn-MnPn, 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. 6. Based on Fig. 6, the negative control group had a hanging time of more than 20 sec, means the mice were catalepsy. Group treated by L-dopa, the extract and Zn-MnPn on the all five doses had an average of hanging time of fewer than 20 sec. Although the hanging time was longer than the normal control group which was 2 seconds, this indicated the intensity of catalepsy in mice of these groups decreased. Fig. 5 also shows that the hanging time of the groups treated by Zn-MnPn on the all five doses was shorter than the group treated with the extract. This showed that Zn-MnPn on the all five doses lowered the catalepsy better than the extract. The hanging time of group treated by Zn-MnPn at a dose of 10 mg/kg body weight was shorter than its of group treated with L-dopa, hence Zn-MnPn at a dose of 10 mg/kg body weight lowered catalepsy better than the positive control group.

![Figure 6. Diagram of catalepsy decrease](image)

A statistical test using one-way ANOVA followed by Dunnett post hoc test was conducted to determine the significance of Zn-MnPn 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. Zn-MPn 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              | Zn-MPn 5 mg/kg | Zn-MPn 10 mg/kg | Zn-MPn 15 mg/kg | Zn-MPn 20 mg/kg | Zn-MPn 25 mg/kg |
|--------------------|---------------|----------------|----------------|----------------|----------------|
| Normal             | 0.047*        | 0.323          | 0.093          | 0.079          | 0.002*         |
| Negative control   | 0.126         | 0.019*         | 0.068          | 0.079          | 0.949          |
| Positive control   | 0.844         | 0.998          | 0.982          | 0.962          | 0.067          |
| The extracts       | 1.000         | 0.613          | 0.983          | 0.994          | 0.336          |

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

Group treated with Zn-MPn at a dose of 10 mg/kg body weight showed the $P$ value less than 0.05 compared to negative control, means the condition of the mice was significantly different with the condition of the mice in negative control group. It demonstrated that Zn-MPn at a dose of 10 mg/kg body weight lowered the catalepsy significantly. This group also showed the $P$ value more than 0.05 compared to normal and positive control groups. This showed that the administration of Zn-MPn at a dose of 10 mg/kg body weight lowered catalepsy until the mice being normal (healthy) and the activity of Zn-MPn at a dose of 10 mg/kg body weight in lowering catalepsy was equal to its of L-DOPA. In the other hand, although the administration of Zn-MPn at a dose of 5, 10, 15, and 20 mg/kg body weight lowered the catalepsy better than the extract, statistically its activity was not significantly different.

4. Conclusion
Zn-MPn has successfully synthesized from zinc acetate dihydrate (Zn(CH$_3$COO)$_2$·2H$_2$O) solution with Mucuna pruriens seed extract for 40 min. The Zn-MPn obtained has a particle size of 55 nm with an oval in shape. Zn-MPn has a typical band of Zn-O interaction in the absorption area of 464.8 cm$^{-1}$ on the FTIR spectrum. Zn-MPn at a dose of 10 mg/kg body weight demonstrated to lower the catalepsy in mice significantly.

5. References
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