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GREEN BIOSYNTHESIS APPROACH OF SILVER NANOPARTICLE USING CRUDE EXTRACT OF *LEMNA* SP.

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Biosynthesis; Silver Nanoparticles; *Lemna* sp; extraction

ABSTRACT

Silver nanoparticles (AgNPs) recently has become an emarging field due to it is vast utilization including antibacterial agent, catalyst, and biomedical aplication. One of problems faced in the synthesize of AgNPs use the of toxic reducing agents which are not environmentally friendly and relatively high cost. In this study we demosntrated a green and Environment friendly Shythesis of AgNPs using *Lemna* sp extract as the reduction agent. *Lemna* sp were extracted using multilevel extraction method using n-hexane, ethyl acetate and ethanol with a ratio of 1:10 (w/v). The crude extract of *Lemna* Sp was added to stirred AgNO3 4 mM solution and left for 2 hours in the dark. The results showed that crude extract *Lemna* sp can reduce AgNO3 from AgNPs were characterized by a change in the color of the solution an increase in absorbance recorded by UV-Vis Spectrofotometer. In general, we succesfully shynthesized AgNPs using extract *Lemna* sp. in a mild condition

INTRODUCTION

Nanoparticles are small particles and very fine nanometer size [1]. Nanoparticles can also be interpreted as microscopic particles measuring 1-100 nm. [2]. Nanoparticles have unique properties such as optical, thermal, chemical, physical, and electricity [3]. Therefore, nanoparticles are widely applied in the fields of coding, chemistry, environment, agriculture, information and communication. Nanoparticles (NPs) are grouped into two main types namely organic and non-organic [4]. Organic nanoparticles are carbon while those included inorganic nanoparticles consist of three types namely magnetic nanoparticles, precious metal nanoparticles (gold, platinum and silver) and semiconductor nanoparticles (titanium oxide and zinc) [5].

There are several ways that can be done to obtain nanoparticles, namely biological and chemical. One way that is often used is biosynthesis. Biosynthesis is a way to synthesize nanoparticles by biological methods that use microorganisms and plant extracts [6]. The biosynthesis process with this method is very efficient, inexpensive, fast reaction time, environmentally friendly and the synthesis process does not use toxic chemicals [7] so that the nanoparticles produced can be utilized in biomedicine and pharmacy. Another development method for synthesizing AgNPs is to usetechniques, green synthesis namely by utilizing natural materials as reducing agents [8]. Natural materials derived from these plants are known to reduce metal ions such as silver ions (Ag^+) to Ag^0 because these plants have chemical components that can penetrate the metal. In this research biosynthesis of silver nanoparticles was carried out using the extract of *Lemna* sp.

Synthesis of silver nanoparticles (NPs) can be done in two ways, namely top to buttom and button to up. This method is called themethod eco-friendlyof synthesis of silver nanoparticles withextract *Lemna* sp as a reducing agent oragent capping. Plant extracts are mixed with a metal salt solution which is carried out at room temperature. The reaction will occur after a while as a result of changes in metal or silver converted from mono or divalent to valence zero which was marked by observed color changes [9]. The process of biosynthesis of silver nanoparticles was also influenced by several things and according to the study of Almeida *et al.,* [10] showed that the concentration of plant extracts became an important factor in determining NP size, incubation time, and pH only slightly affect morphology. Meanwhile, the temperature did not show a significant effect either in the size, morphology or structure of the nanoparticles produced. In addition, it is suspected that nanoparticle biosynthesis also involves secondary metabolites from plants, such as flavonoids and triterpenoids [11].

In this study, the extract used to synthesize silver nanoparticles was *Lemna* sp. *Lemna* sp is a type of monocot plant that lives freely and is commonly found on the surface of water. *Lemna* sp is also included in the family *Lemna*ceae which can grow at temperatures of 60C-330C with an optimum pH of 5.5-7.5 [12]. *Lemna* sp also includes aquatic plants that are found and grow on the surface of the water with a very wide spread rate. This plant is known as weed because it is sometimes difficult to control. However, this plant is rich in protein and nitrogen so that it can reduce various types of metals and is used as phytoremidiation [13]. Heavy metals that can be reduced in Cd, Hg, Zn, Mn, Pb, and Ag [14].

MATERIALS AND METHODS

Research methods

This research was conducted through direct experiments and carried out in biology laboratories, Food technology laboratories and Chemical Laboratories for 3 months from January to March 2020. The extraction method employed was stratified extraction with n-hexane, ethyl acetate and ethanol, while the silver nanoparticle biosynthesis method was *Bottom-up.*

Extraction *Lemna Sp*

Lemna sp. *was* dried for 4 days then mashed and weighed as much as 25 grams. *Lemna sp* 25 grams were then dissolved in 250 mL n-hexane and extracted for 3 days in a *watterbaht shaker* 112 Rpmat 28^0C. After 3 days extraction of the filtered solution resulted in extracts and pulp. The resulting pulp is extracted using ethyl acetate and then ethanol. The extract obtained was separated from the solvent using a *rotary evaporator.* The sample obtained was centrifuged at 5000 rpm with a temperature of 24^0C for 15 minutes then divortexed and diluted to 10dilution^{-2} (Fig. 1)

Biosynthesis of silver nanoparticles The

Extract to be biosynthetic was extracted *Lemna sp* with ethanol solvent. A total of 18 mL of 4 mM AgNO3 was added to Erlenmeyer and 2 mL of extract was added. Biosynthesis is carried out for 24 hours and then the absorbance were recorded with sectrophotometer at wavelengths of 300-600 nm with intervals of 50 nm.

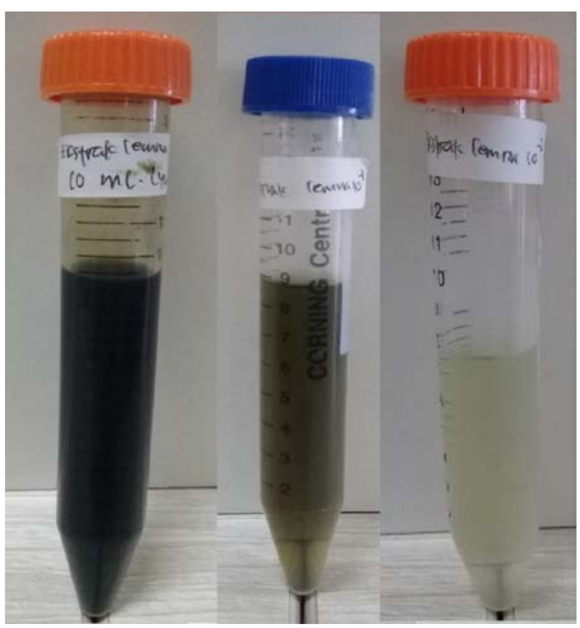

Fig. 1. Dilution of *Lemna* sp. 10^0, 10^{-1}, 10^{-2}

RESULTS AND DISCUSSION

The extract produced from each solvent used has a different color where the ethanol solvent filtrate has a more concentrated color (fig. 2). The volume of filtrate produced from each extract in the same ratio also varies from one to another. The highest volume of filtrate is the filtrate with ethyl acetate solvent (Table.1).

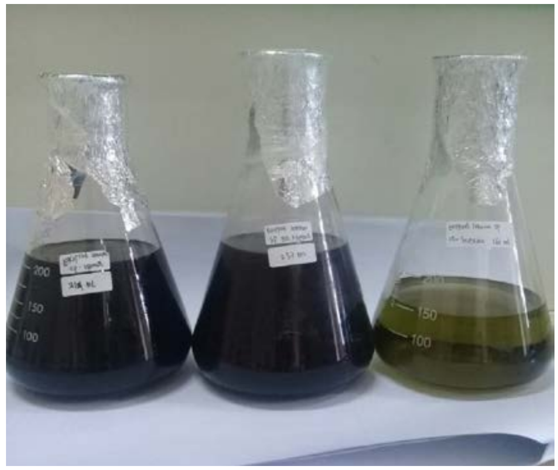

Fig. 2. Extract Filtrate *Lemna sp* (from left-right: ethanol, ethyl acetate, and *n*-hexane)

Table. 1. Results of Extraction each Solvent

No	Solvent	Weight	Solvent Volume	Filtrate Volume
1	*n*-heksana	25 gram	250 mL	161 mL
2	Etil Asetat	25 gram	250 mL	237 mL
3	Etanol	25 gram	250 mL	214 mL

Based on the results of research conducted silver nanoparticle synthesis successfully carried out which is characterized by changes in the color of the solution from clear to yellowish and more turbid which can be observed directly and changes in absorbance values over time time during the synthesis process. Basically an increase in the absorbance value of the synthesis process occurs quickly just after the sample is added to the AgNOsolution$_3$. However, the increase and decrease in absorbance values become unstable. This is caused by several things, including the small sample period and the formation of nanoparticles that are not perfect into larger particles (in the form of aggregates) which are characterized by the presence of sediment at the bottom of the bottle. Aggregation formation is also caused by unstable NPP so NPP will merge and form NPP no longer in nano form. Therefore, the absorption of light spectrophotometers can also be imperfect. At the time of synthesis of silver nanoparticles, thenot added *stabilizer was* so that it was possible to form nanoparticle aggregates because according to Haryono, et.al (2008) said that the process of synthesis of silver nanoparticles tends to aggregate into a larger size so the addition of *stabilizers* becomes very important for avoid the aggregation of nanoparticles. *stabilizers* that can be added are in the form of polymers such as poly vinyl alcohol (PVA), poly vinyl pyrrolidine (PVP), poly ethylene glycol (PEG), poly styrene sulfonate (PSS), poly acrylic acid (PAA) and chitosan [15].

Based on Figure 3 and Table. 2 shows that changes in absorbance values over time during the nanoparticle synthesis process have increased although there has also been a decrease. This shows that the process of forming silver nanoparticles has been successfully carried out. Apart from that, from the it is seen that the most optimum NPP produced is characterized by the highest absorbance at a wavelength of 450-500 nm with a conical single peak (Table 2). This is in accordance with research conducted by [16] which shows that the most optimum NPP increase occurs at a wavelength of 400-500 nm.

Figure 3. Drying of *Lemna* sp (top: HI, H-2. Bottom: H-3, H-4)

Table 2. Spectrum of biosynthesis of silver

Absorbance	t(0)	t(1)	t(2)	t(3)	t(4)	t(18)	t(20)	t(22)	t(24)
300 nm	0.013	0.17	0.196	0.272	0.226	0.218	0.687	0.196	0.189
350 nm	0.061	0.103	0.122	0.169	0.134	0.114	0.308	0.101	0.107
400 nm	0.106	0.112	0.126	0.157	0.13	0.12	0.153	0.114	0.106
450 nm	0.041	0.059	0.077	0.107	0.085	0.086	0.11	0.181	0.075
500 nm	0.033	0.036	0.48	0.072	0.053	0.053	0.082	0.052	0.045
550 nm	0.03	0.028	0.039	0.059	0.04	0.037	0.065	0.04	0.033
600 nm	0.029	0.029	0.036	0.054	0.037	0.033	0.06	0.03	0.027

Conclusion

Based on the results of research conducted synthesis of silver nanoparticles successfully carried out using the extract of *Lemna* Sp. which is characterized by changes in the color of the solution from clear to yellowish and more turbid which can be observed directly and changes in absorbance values over time during the synthesis process. Therefore, *Lemna* Sp. can be a reducing agent for Ag + because it has a high protein and nitrogen content. *Lemna* Sp. also widely used as a metal phytoremidiation agent, one of which is silver. The most optimum NPP produced is characterized by the greatest absorbance at a wavelength of 450-500 nm which is characterized by a conical single peak.

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