

# Synthesis of Silver Nanoparticles Balaccida Leaf Extract and Its Anti-Bacterial Activity of E. Coli

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## Article Info

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## ABSTRACT

The Covid-19 pandemic has become a major problem for public health around the world. One of the ways to prevent Coronavirus is by maintaining hand hygiene using hand sanitizer. The main ingredient of most antiseptics circulating in Indonesia is alcohol which can cause skin irritation. Alcohol substitutes as antiseptics in hand sanitizers are widely available in nature, one of which is the balakacida plant (*Chromolaena odorata*). The availability of balakacida plants in Indonesia is very abundant as wild plants are often referred to as nuisance plants (weeds). The phytochemical content of balakacida leaves includes saponins, flavonoids, tannins, and alkaloids that have the potential as antibacterial. Balakacida leaf extract has the potential to be used as a source of active ingredients in antiseptic preparations as an alternative to maintain hand hygiene from various parasitic microorganisms. AgNPs are one of the antibacterial and antifungal agents that are effective in inhibiting and killing microorganisms. The optimal variation of CO-SNPs was found at 3 ml of CO-SNPs with an absorption peak of 409 nm and a particle size of 230.1 nm. CO-SNPs produced the best inhibitory value of 12.26 mm on E.coli bacteria.

**Keyword:** CO-SNPs, antiseptics, balakacida, *chromolaena odorata*

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## 1. INTRODUCTION

The Covid-19 pandemic is currently a major problem for public health around the world. The disease caused by a new type of coronavirus named SARS-CoV-2 was first spread in Wuhan City, China in December 2019, and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (WHO, 2021). The Covid-19 Handling Committee and National Economic Recovery (KPCPEN) reported that provincial data from the Indonesian Ministry of Health until October 10, 2021, the distribution of Covid-19 cases reached 4,220,206 confirmed cases, including 31,054 active cases, 4,046,891 recovered, and 142,261 died. Currently, there is an increase in Covid-19 cases ranging from 10-15% every week.

Coronavirus is a virus that causes respiratory tract infections in humans and animals. The spread of the virus can be through human hands which are often contaminated with microbes. Virus prevention can be overcome by implementing the Health protocol, namely 5M, one of which can be through maintaining hand hygiene. Hand hygiene is also very necessary in the field of microbiology and places that are prone to the spread of microorganisms through hand media. One of the main steps to prevent the Covid-19 virus is to clean hands with antiseptic (WHO, 2021).

Hand sanitizer is an antiseptic liquid that is generally used to kill infectious agents on hands. The use of hand sanitizer is also more practical than washing hands with soap, especially for people with high mobility and cannot find a hand-washer around them. Currently, the main ingredient for most hand sanitizers circulating in Indonesia is alcohol (Fibriana, Amalia, Muntamah, Ulva, & Aryanti, 2020). The main ingredient is expected to be able to kill bacteria, viruses and fungi. However, the addition of alcohol with a high percentage (60-90% ethanol) in an antiseptic that is used with a short frequency will certainly cause skin dehydration and skin irritation (Auliasari, Rantika, & Yuliarti, 2017) because alcohol is hygroscopic and hydrosylate. The need for the use of local herbal-based antiseptics is expected to be able to replace alcohol-based antiseptics.

Indonesia is one of the countries with the greatest biodiversity richness in the world which has more than 30,000 species of higher plants (Jamilah & Disemadi, 2020). One of them is Balakacida (*Chromolaena Odorata*) which is a type of plant from the Asteraceae family, which mostly grows in subtropical and tropical areas. The availability of Balakacida plants in Indonesia is very abundant as a wild plant in gardens, roadsides and rice fields. The existence of Balakacida plants is often referred to as a nuisance plant (weed) in the form of a multi-stemmed bush so that it is often destroyed.

The very fast growth of Balakacida causes damage to cultivated plants, so that it can harm farmers. Currently, some people use Balakacida as a live pharmacy plant to cure various diseases. Some of the uses of this plant include medicine for bleeding wounds, new wounds, and treating diseases caused by bacterial infections. The parts that are widely used are the leaves and flowers of Balakacida (Isman, Andani Ahmad, & Abdul Latief, 2021).

The content of phytochemicals from the leaves of the Balakacida plant (*Chromolaena odorata*) includes saponins, flavonoids, tannins, polyphenols, terpenoids, and essential oils (Jagatheesh et al., 2020) which have potential as antibacterial. Research conducted by (Sari Wahyu, 2020) and states that Balakacida leaf extract has a fairly high antibacterial activity as an antiseptic, making it possible to be used as protection against the dangers posed by parasitic microorganisms such as bacteria and viruses that can interfere with the human immune system.

Balakacida leaves (*Chromolaena odorata*) have the potential to be used in the pharmaceutical field as a source of active ingredients in topical antiseptic spray preparations as an alternative to maintaining hand hygiene from various parasitic microorganisms by extracting the Balakacida leaf parts and then making preparations based on research that has been done. In a study conducted by (Febrianasari, n.d.) leaf extract of the Balakacida plant (*Chromolaena odorata*) had an effective inhibition against *S. aureus* bacteria at a concentration of 100% of 7.47 mm.

Darsanasiri, et al (2013) stated that one of the effective ingredients as antibacterial and antifungal agents in inhibiting and killing fungal microorganisms is silver nanoparticles (AgNPs). Currently the use of AgNPs is widely used in electronic industrial applications, also acts as an antibacterial agent which is used for various household applications to water treatment and sterilizing medical devices (Yaqoob, Umar, & Mohamad Ibrahim, 2020). However, the use of AgNPs for antiseptics has not been.

## 2. RESEARCH METHOD

The type of research used is experimental research. The subject and object of this research is the synthesis of balakacida leaf extract with AgNPs, there are variations in the concentration of balakacida extract 1%, 3%, and 5% and observe the activity and characteristics of the synthesis of balakacida leaf extract with AgNPs against *E. coli* bacteria. This research method uses the green chemistry method by bottom-up synthesis of AgNPs. This method is more effective because it is environmentally friendly and economical.

### 2.1. Making Balakacida Leaf Extract

Preparation of balakacida leaf extract (*Chromolaena odorata*) using maceration method for 1x24 hours with 96% ethanol solvent. Furthermore, the filtrate from the maceration was evaporated using a Rotary Evaporator at a temperature of 70°C to form a thick extract.

## 2.2. Phytochemical Test

Phytochemical tests were carried out to identify the content of active compounds in the form of flavonoids, saponins, and tannins contained in balakacida leaf plants. Flavonoid test was carried out using a test tube as much as 5 ml of balakacida leaf extract was heated to boiling then added 5 drops of concentrated HCl and added 0.2 gram of Mg powder. The content of flavonoids is indicated by a change in color to yellowish brown, orange or brownish red. The saponin test was carried out using a test tube as much as 0.5 g of balakacida leaf extract added with 5 ml of distilled water and shaken very vigorously. The content of saponins is indicated by the presence of foam in the solution. Tannin test was carried out using a test tube of 0.5 g of balakacida leaf extract plus 2 ml of 70% ethanol. Strain and add a few drops of 1% FeCl<sub>3</sub> until it changes color. Tannin content is indicated by the color turning blue-black or greenish-brown.

## 2.3. Synthesis of Silver Nanoparticles with Balakacida Leaf Extract (CO-SNPs)

The synthesis of CO-SNPs was carried out using the green chemistry method. The steps for the synthesis of CO-SNPs were Balakacida leaf extract (1%, 3%, and 5%) slowly added to a solution of AgNO<sub>3</sub> (0.001M) in a 250 mL Erlenmeyer flask. The solution in the Erlenmeyer flask was stirred using a magnetic stirrer for 30 minutes in a dark room to reduce Ag<sup>+</sup> to Ag<sup>0</sup>. Then the sonication process was carried out using an ultrasonic cleaner for 15 minutes to reduce the agglomeration process and reduce the size so as to obtain the optimal particle size. The color change to dark brown indicated the formation of AgNPs synthesis with the extract.

## 2.4. MHA (Mueller Hinton Agar) Media Production

MHA was dissolved in an Erlenmeyer flask with distilled water up to the mark and then homogenized using a stirrer. The media was then sterilized using an autoclave for 15 minutes at 121°C. The media was then poured into a petri dish as much as +/-25 mL and allowed to solidify.

## 2.5. Making NA Media (Nutrient Agar)

The manufacture of NA media was used to breed E. coli bacteria. NA was dissolved in distilled water and then homogenized with a stirrer and heated using a hot plate, then sterilized by autoclaving, so that sterile NA media was obtained.

## 2.6. Bacterial Culture

Bacterial culture is carried out with the aim of rejuvenating and multiplying bacteria. Cultures of E. coli bacteria in slanted agar were inoculated aseptically into MHA as much as 1 ose and then incubated for 24 hours at 37°C.

## 2.7. Bacterial Inhibition Test

Adapted from Octaviani et al., (2019), the bacterial inhibition test against antiseptic preparations was carried out using the disc diffusion method. The test bacteria E. coli was inoculated on MHA media in a petri dish using trigalski. Furthermore, the blank disc was immersed in the three extract variations, each with a positive control of chloramphenicol and a negative control of ethanol tested in three repetitions. Then the blank disc was placed on the inoculum and incubated for 24 hours at 37°C. Furthermore, observations and measurements of the diameter of the clear zone formed around the blank disc were made using a caliper.

## 2.8. Data Analysis Technique

The data analysis technique used UV-Vis analysis and PSA analysis. In UV-Vis analysis by observing the color change in the solution and measuring the UV-vis spectrum with a wavelength range between 300 to 700 nm. The reaction between Ag<sup>+</sup> and balakacida leaf extract can be observed in the UV-vis spectrum. The PSA test was performed to analyze the particle size of the synthesis of AgNPs of balakacida leaf extract with a particle size of 1-100 nm using the SYMPATEC HELOS-BF Laser Particle Size Analyzer.

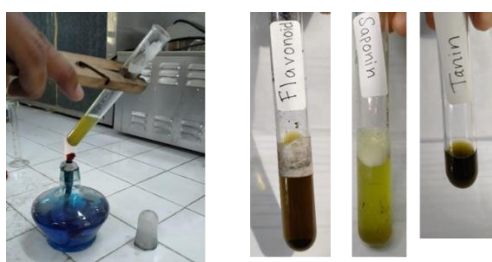
### 3. RESULTS AND ANALYSIS

#### 3.1. Balakacida leaf extraction

Extraction begins by weighing 250 grams of balakacida leaf powder which has been dried for 3x24 hours under the hot sun and mashed using a blender. Then extracted using 96% ethanol solvent by maceration method for 1x24 hours. The filtrate from the maceration was evaporated using a Rotary Evaporator at a temperature of 70°C and 5 grams of thick extract was produced. Balakacida leaf extraction aims to extract secondary metabolites in the form of flavonoids, saponins, and tannins and eliminate other compounds that are not used.

#### 3.2. Balakacida Leaf Extract Phytochemical Test

Based on the results of phytochemical tests conducted by researchers, balakacida leaf extract contains secondary metabolites in the form of flavonoids, saponins, and tannins as evidenced by.

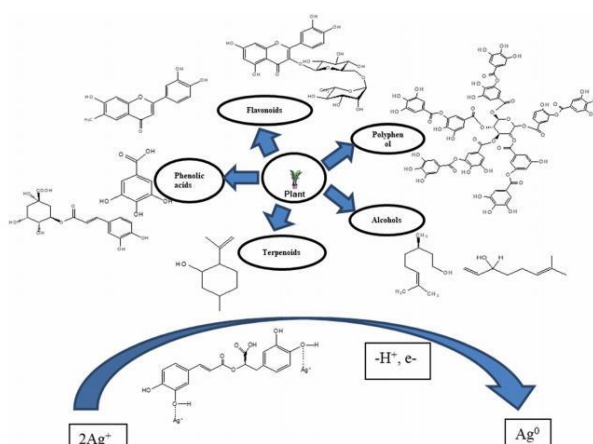


**Figure 1.** Balakacida leaf extract phytochemical test results

In the test of flavonoid compounds by heating 5 ml of balakacida leaf extract to a boil, then adding 5 drops of concentrated HCl and 0.2gram of Mg powder. Based on the observations of the researchers, there was a change in the color of the solution to yellowish brown, this indicates the positive balakacida leaf extract contains flavonoid compounds. Testing of saponin compounds was proven by inserting balakacida leaf extract into a test tube then shaking vigorously and foaming or foaming was formed. Tannin compounds were proven by mixing balakacida leaf extract with 2 ml of ethanol then adding 2-3 drops of 1% FeCl<sub>3</sub> solution and the color changed to greenish brown.

#### 3.3. Synthesis CO-SNPs

The synthesis of CO-SNPs was carried out with variations of balakacida leaf extract as much as 1, 3, 5 ml. The results of the synthesis of silver nanoparticles of CO-SNPs are characterized by a color change to dark brown and have a spectrophotometer absorption value at 400-450 nm. In this study, the three variations of the sample produced a brownish green color which indicated the process of reducing Ag<sup>+</sup> ions to Ag<sup>0</sup>.



**Figure 2.** Synthesis AgNPs

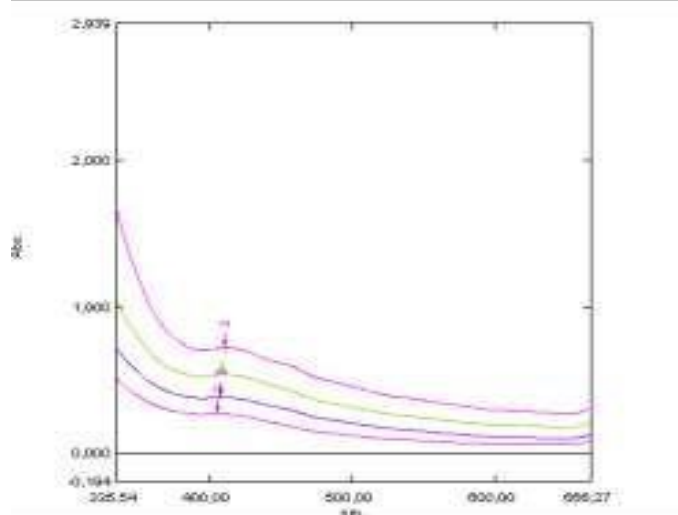
In addition, there is a green deposit at the bottom of the Erlenmeyer. The higher the concentration of CO-SNPs, the more precipitate formed. The extract was filtered once and needed to be filtered again. The following are four samples of CO-SNPs that will be characterized by UV-Vis and PSA after 10 times dilution.



**Figure 3.** Sample of CO-SNPs

### 3.4. UV-Vis Analysis

The synthesis of silver nanoparticles was indicated by the presence of an absorption spectrophotometer at 400-450 nm.



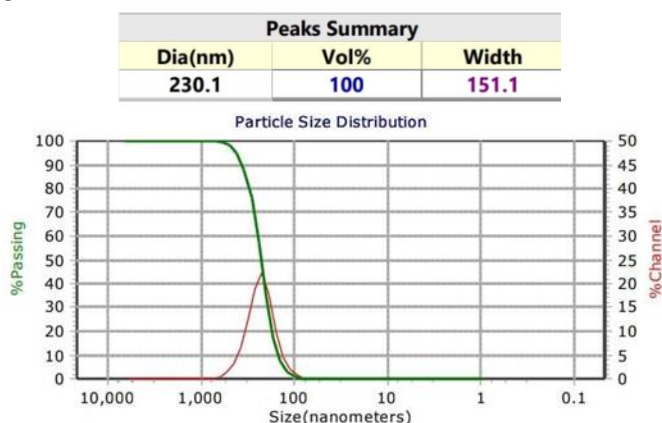
**Figure 4.** Graph of UV-Vis Test Results

The UV-Vis test with a spectrophotometer 2450 showed that the three samples had the first absorption peak in the 600nm range but the second absorption peak in the 400 nm range. The high absorption peak value was due to UV rays not being able to penetrate the cuvette well due to the presence of green deposits floating on the cuvette. In addition, the high absorption value was caused by agglomeration in the CO-SNPs solution which caused the particle size to become large.

At 3 ml CO-SNPs had a second peak with an absorption peak of 409 nm, 1 ml CO-SNPs had an absorption peak of 411 nm, and CO-SNPs 5 ml had no absorption peak in the 400-450 nm range. This is supported by the absence of a reduction process and color change in the 5 ml CO-SNPs. In the figure, CO-SNPs 1 and 3 ml have a non-sharp peak and several other peaks. This is due to the non-uniform particle size of CO-SNPs. In addition, the high absorption value was caused by the agglomeration of CO-SNPs which caused the particle size to become large. Basically silver nanoparticles have a tendency to agglomerate, so a stabilizer is needed to prevent agglomeration.

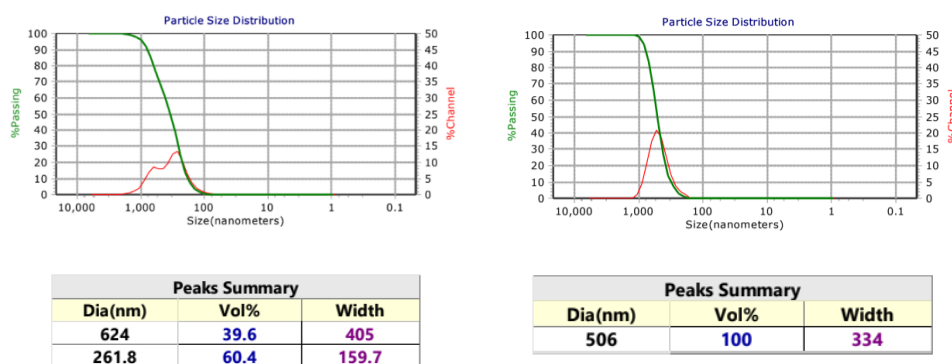
### 3.5. PSA Analysis

The PSA analysis was performed to analyze the particle size of the synthesis of AgNPs of balakacida leaf extract using the SYMPATEC HELOS-BF Laser Particle Size Analyzer. The result of analysis is shown in Figure 5 and 6.



**Picture 5.** PSA CO-SNPs Test Results 3%

Based on the PSA test that has been carried out, the best particle size was obtained at 3 % CO-SNPs with a diameter value of 230.1 nm.



**Picture 6.** PSA CO-SNPs Test Results 1% and 5%

While the CO-SNPs 1 and 5 ml have a size of about 600 nm with a low level of solution homogeneity. The low size homogeneity was caused by the non-uniform particle size, but also due to the non-optimal reduction process due to excess extract volume. Silver nanoparticles have optimal sizes in the 1-100nm range. Basically silver nanoparticles have a tendency to agglomerate, so it is necessary to have a stabilizer with an optimal concentration to prevent agglomeration.

### 3.6. Bacterial Inhibitory Test

Bacterial inhibition test was carried out using the synthesis of silver CO-SNPs nanoparticles with variations of balakacida leaf extract 1, 3, and 5 ml on E.coli bacteria. The table below shows that the results of the best inhibitory test on the synthesis sample of balakacida leaves at a concentration of 3ml are 12.26 mm. Meanwhile, in the three variations of silver nanoparticles and AgNO<sub>3</sub> samples, the average results were relatively the same, meaning none. This is because the three variations of silver nanoparticles have not yet produced a nano size (0-100nm). The results of the bacterial inhibition test is shown in Figure 7 below.

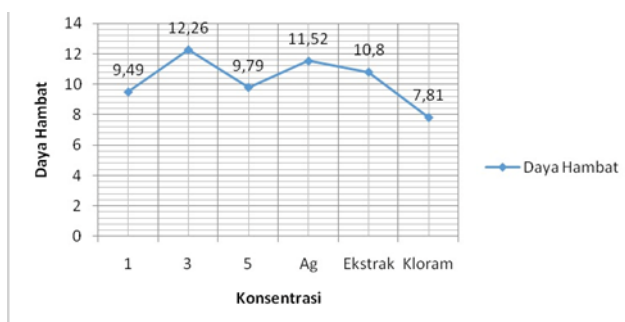


Figure 7. Bacterial inhibitory test graph

#### 4. CONCLUSION

The synthesis of Balakacida Leaf Extract (*Chromolaena odorata*) with silver nanoparticles of CO-SNPs obtained optimal variation at 3 ml of CO-SNPs with an absorption peak of 409 nm and a particle size of 230.1 nm. CO-SNPs produced the best inhibitory tilapia of 12.26 mm on *E.coli* bacteria. This research can be developed by adding a stabilizer to CO-SNPs which aims to form a reduction process and prevent agglomeration in order to obtain nanoparticles with a particle size of 1-10 0nm.

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