



## Utilization of Nanogold and Nanosilver as Drugs Delivery on Antioxidant Activity in Herbal Medicine Preparation During Pandemic Covid-19

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**ABSTRACT:** The purpose of this study was to determine the effect of adding nanogold and nanosilver with various concentrations on the antioxidant activity of herbal medicine made from black seed, black garlic extract, and propolis. The concentration of nanogold used was 5,10,15,20,25 and 30 ppm with 20 ppm nanosilver concentration. In this study, the activity of antioxidant compounds was tested using the DPPH method with a UV-Vis spectrophotometer. This study shows the percentage of free radical reduction results. The results of this study indicate that there is an effect of adding nanogold in the antioxidant content of herbal medicines. The best nanogold concentration was shown at a concentration of 30 ppm, which was 84,42%. The results show that the greater the concentration of nanogold added, the greater the percent reduction.

**KEYWORDS:** Antioxidant activity, Herbal medicine, Nanogold, Nanosilver.

### INTRODUCTION

One of the efforts to prevent contracting the virus that can be done is to increase body resistance and health. This virus can enter the body through droplets or fluids that are released through the mouth, nose, and eyes. The molecular biology institute has informed that there is a possibility of spreading the coronavirus through the air in the form of aerosols, however this only happens a lot in hospitals because some medical procedures performed in the use of ventilators can produce aerosols with a smaller number of the particle so that the spray distance becomes longer. He stated that if a person is contaminated with droplets with the corona virus, the virus will enter the lungs through the respiratory tract or the virus can also enter the intestines through the eyes [1].

The principle of increasing immunity to fight virus can be done in several ways including increasing the body's foremost defense (natural immunity) owned by the body, stimulating the production of immunoglobulin in the circulation, blocking the virus from binding to receptors, reducing the intensity of cytokine storms and decreasing the speed of replication virus [2]

The body's resistance can be maintained and increased by adopting healthy living habits such as maintaining a clean environment, increasing the intake of good nutrition, and using health supplements and herbal medicinal ingredients [3].

The ingredients of herbal medicines are black cumin (haubatussauda), propolis and extract black garlic. Because black cumin extract has anti-diabetic, anti-hyperlipidemic, anti-ulcer, anti-hypertensive, and antineoplastic activities this can strengthen scientist claims that black cumin can help reduce the coronaviruses that enter patients with comorbid conditions. In addition, the active compounds nigellidine and hederin have also been identified to help inhibit the potential for SARS CoV-2 [4].

Propolis This medicine has a bitter taste but is very efficacious to relieve various complaints directly such as coughs, colds, and shortness of breath to be relieved. . Propolis extract can reduce free radical exposure by 50%. So it is suspected that the phenolic compounds contained in propolis play an active in binding free radicals [5].

In 2019, a trial of this herb was carried out together with nanogold nanosilver volunteers with leprosy. Apart from acting as a strong antioxidant, nanogold also acts as drug delivery of the active medicinal ingredients found in haubatussauda and propolis, so that the efficacy of both is even greater, especially in increasing the body's immunity

There is a natural immunity called glutathione in the body, which is located in the cells. As a protein that is naturally produced in the body, glutathione has three functions that are important protection as an antioxidant, immune system booster, and detox [6]. The addition of nanogold in herbal medicine can help glutathione to maximize forming more effective clusters. Glutathione molecules that synergize with nanogold can form a cluster that can improve the performance of endogenous antioxidant content. The strength of this collaboration is needed especially when dealing with a pandemic or outbreak and beyond.



Research has been carried out on nanogold and nanosilver which states that the antioxidant activity of nanosilver tested by the DPPH method shows that nanogold has been shown to have antioxidant activity because it can reduce exposure to DPPH free radicals. Nanogold activity is evidenced by reacting with free radicals on DPPH. Nanosilver also has properties that are almost the same as nanogold, namely by being synthesized from precious metals, biocompatible and antioxidant because it can reduce and capture free radicals [7]

According to research, nanogold has properties that can increase stamina and balance the production of hormones in the body that are useful for rejuvenating cells in the body. Meanwhile, the antiviral activity contained in nanosilver can also be used to support antivirus updates, especially for viruses that are indicated by flu symptoms. Nanosilver properties have so that bioavailable it can safely and is consumed in the form of herbal medicinal liquids.

The toxicity test results of nanogold and nanosilver show that nanogold and nanosilver are safe for consumption by the body. Nanogold is also believed to be able to inhibit the process of infection that occurs in the body caused by viruses. A combination of medicinal ingredients herbal medicines with nanogold and nanosilver tested positive can affect several diseases. Nanogold and nanosilver positively affect patients' quality of life who have diseases caused by viruses [8].

Further antioxidant activity tests were carried out using the DPPH method to determine antioxidant activity. This test is based on the content of hydroxyl groups which will cause the number of compounds to be soaked with DPPH so that the antioxidant activity will be higher. Antioxidant compounds can stop and break the bond of free radical reaction which Antioxidants can donate one or more electrons so that free radicals can be overcome by scavenging [9].

The principle of the antioxidant activity test is to measure the scavenging of DPPH free radicals by an antioxidant compound using a UV-Vis Spectrophotometer and then the value of free radical immersion activity will be known which is expressed in percent immersion [10].

Free radicals are found in the body during activities such as during the process of cellular respiration or the process of burning metabolism in cells. Free radicals can be defined as molecular atom that has a fairly high reactivity [11].

The mechanism of the antibacterial nanosilver starts from the particle that will stick to the surface of the bacterial and then their nature changes into a membrane. Furthermore, the nanosilver attached to bacterial will cause DNA damage which will then be followed by the solubility of nanosilver, in this process nanosilver will release silver ions which have antimicrobial properties which can also interact with protein groups containing thiol compounds in cell walls and at the same time. The same will also affect its function [12]

Free radicals also have unstable properties, this happens because a free radical contains one or more lone pairs of electrons in its outer shell, this causes a compound to reach a stable form so that it will become an unreactive radical [13].

## METHOD

### Tool

The tools used in this research are measuring cylinder, dropping pipet, volumetric flask, filter paper, beaker, spatula, Erlenmeyer flask, measuring flask, funnel glass, analytical balance, test tube, test tube rack, UV-Vis spectrophotometer, and TEM.

### Material

$\text{HAuCl}_4$  1mM, Sodium Citrate, Herbal Medicine, aquades, 96% ethanol, 1000 ppm  $\text{AgNO}_3$  Solution and DPPH

### Procedure

#### Nanogold Synthesis

200ml of distilled water was put in a beaker glass then heated to boiling, then added 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL, and 3.5 mL  $\text{HAuCl}_4$  1000 ppm in each beaker containing boiling distilled water. After that, each added 0.3 grams of sodium citrate. Then stirred until homogeneous and left until the color changes to wine red [10].

#### DPPH Solution Preparation

0.004% DPPH solution was prepared by dissolving 4 mg of powder DPPH is put into 100 measuring pumpkins mL, then added ethanol up to the mark and then shaken until homogeneous and dark purple in color. After that, it was stored in a dark place and the DPPH solution was measured with a UV-Vis spectrophotometer at a wavelength of 400-600 nm. So that the maximum DPPH data is obtained which will be used to measure the absorbance of the sample [10].

**Qualitative Antioxidant Analysis**

1mL of the herbal medicine sample solution was put into a test tube and then 4mL of 0.004% DPPH solution was added little by little and the color changes were observed. The presence of antioxidant compound activity in herbal medicinal syrup preparations can be characterized by the formation of yellowish color in the test sample [14].

**Test the Effect of Addition of Nanogold on Antioxidant Activity of Herbal Medicines**

3 ml of herbal medicine samples were taken and put into 6 dark bottles and then 3 ml of the solution was added nanogold at concentrations of 5, 10, 15, 20, 25, and 30 ppm and added 6 mL of 0.004% DPPH solution. Then 2 ml of 20 ppm nanosilver solution was added. Then the solution was shaken and allowed to stand for 30 minutes. Then The absorption wavelength was measured using UV-Vis spectrophotometry at max DPPH. The absorbance and were recorded calculated % of free radical reduction [10]

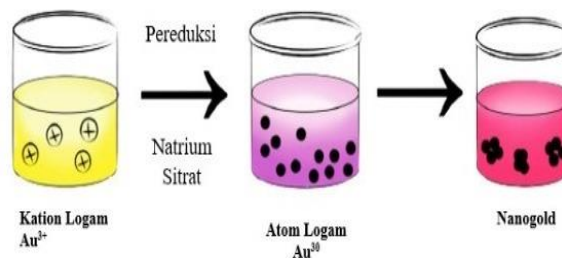
**RESULT AND DISSCUSION**

**Nanogold Synthesis**

The synthesis of nanogold was carried out by applying the chemical method (bottom-up). The basic ingredients used in the nanogold synthesis process are  $\text{HauCl}_4$  with a concentration of 1000 ppm, sodium citrate, and aquades. The addition of sodium citrate compounds is used as a goal for the reduction of gold metal ions ( $\text{Au}^{3+}$ ) contained in the initial  $\text{HauCl}_4$  compound into a gold atom that has no charge ( $\text{Au}^0$ ) [15].

According to the theory, gold compounds that are not charged ( $\text{Au}^0$ ) in a certain amount can easily combine and form a cluster that will continue to experience changes in the magnification of the cluster that has nano size. Besides being a reducing agent, sodium citrate can also act as a stabilizer for nanogold, the net ion contained in the sodium citrate compound will surround the surface of the nanogold so that the nanogold will be more difficult to undergo the aggregation process [10]

The reaction mechanism of the nanogold synthesis process is:  $2\text{Au}^{3+} + 4\text{C}_6\text{H}_5\text{O}_7^{3-} + 6\text{H}_2\text{O} \rightarrow 2\text{Au} + 4\text{C}_6\text{H}_8\text{O}_7 + 3\text{O}_2$  [16].



**Figure 1.** Illustration of Nanogold Synthesis Results

In the nanogold synthesis process, there is a color change from a yellow solution to a colorless solution, this is because there has been no interaction between the Au atoms, then the color changes to a purplish red slowly, this happens because of the interaction between the clusters. When the cluster has a size nanometer then the solution changes color to be burgundy. This color change occurs because during the nanogold synthesis process it indicates the growth of the growing cluster. The following is the result of the nanogold synthesis solution which has a different purplish-red color according to their respective concentrations as shown in Figure 2.



**Figure 2.** Synthesis of nanogold with various concentrations of 5,10,15,20,25 and 30 ppm

Then the results of the nanogold synthesis were characterized by using the Shimadzu 1800 brand UV-Vis Spectrophotometer instrument with a wavelength of 400-600nm and different wavelengths were obtained at each concentration. The maximum wavelength absorbance data can be used to calculate the diameter of the nanogold cluster using the cluster diameter formula (Brus cluster) which is shown as follows:

$$\frac{1240,6}{\lambda} = 1,3 + \frac{14,84}{R^2} \left( \frac{1}{me^2} + \frac{1}{mh^2} \right) - \frac{2,6}{6,5R} \quad [17].$$

From these formulas and data, the wavelength and absorbance results are obtained as shown in the following table:

**Table 1.** Measurement Results of 20 Ppm Nanogold in Various Variations

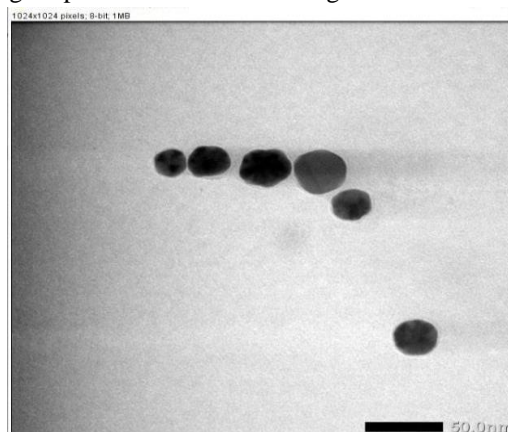
No.	Cluster	Size (nm)	
		1	2
1	1	21.918	20.145
2	2	22.363	21.398
3	3	22.226	22.974
4	4	23.038	25.585
5	5	29.693	23.017
6	6	35.750	21.100

From the results of the data above, it is obtained that the size of the cluster diameter of nanogold particles with a concentration of 20 ppm in the range of 20,145 - 35,750 nm. This shows that nanogold particles can be absorbed by the human body [15].

The TEM (Transmission Electron Microscopy) instrument is one of the instruments with good standards in characterizing a nanoparticle. TEM is also an electron microscope with a working method similar to a slide projector, where a state of electrons penetrates the object. observations and observations can be made with results that are penetrated on the screen [18].

Characterization using the TEM instrument can be used to determine the size of a particle and its distribution. Particles with a size of several nanometers can still be observed using the TEM instrument because the instrument has a very high resolution. The use of high resolution in TEM can assist observations in observing and determining a location where the atoms in the sample are located [18].

The following is an image of the nanogold particles carried out using the TEM instrument.



**Figure 3.** The size of AuNP with a concentration of 20 ppm from the TEM projection

The results of the synthesis of AuNP can be affected by the addition of sodium citrate. The more concentration of sodium citrate added, the more AuCl<sub>4</sub><sup>-</sup> which will be reduced to Au<sup>0</sup> with a very small size. Because in this phenomenon sodium citrate acts as a reducing agent and can also act as a stabilizer [19].

### Synthesis Nanosilver

The synthesis of nanosilver was carried out using the bottom method using 1000 a solution ppm  $\text{AgNO}_3$  with sodium citrate as a reducing agent. The results of the nanosilver synthesis produce a solution that is brownish yellow when the heating process is carried out continuously and is not balanced with the addition of the matrix, the cluster development will continue to increase so that it will form aggregates and will cause silver deposits [11].



Figure 4. Synthesis of Nanosilver with a concentration of 20 ppm

The synthesis of nanosilver has similarities with the synthesis of nanogold, the similarity is that it is both synthesized from metal compounds that have biocompatible properties and contain antioxidants because they can reduce free radicals. Nanosilver also has antimicrobial activity which can also act as an antiviral. In its use, nanosilver which has been used as an antimicrobial can kill about 650 microorganisms that can cause infection [20].

The use with a concentration of 20 ppm is stated to be able to help accelerate the healing of a disease suffered such as herpes and HSV virus metabolism can also be defeated by the presence of antiviral activity in nanosilver that works deep into the cell walls and nucleus of a microbe. That way the cell nucleus of a microbe will be easier to destroy and no one can apply return [21].

### Herbal Medicine Antioxidant Activity Test

Preparation of herbal medicine containing natural herbal ingredients Haubatussauda, Black Garlic, and Propolis. Each sachet consists of two pieces of 5ml ready-to-drink liquid. This medicine has a very bitter taste but is very efficacious to relieve complaints such as coughs, colds, and shortness of breath. The added nanogold and nanosilver can increase antioxidant activity and can also act as a drug delivery agent from the active ingredients found in Haubatussauda, Propolis, and Black Garlic so that the efficacy of both is even greater.

The antioxidant activity test of herbal and nanogold drugs was carried out by measuring the absorbance value of herbal medicines, herbal medicines mixed with pure nanogold and nanogold against a 4% DPPH solution using a UV-Vis Spectrophotometer instrument. The drug solution was made with a concentration of 12.5% and 25% percent of the initial concentration so that a clear yellowish color solution was obtained.

The process of dilution of herbal medicines is carried out using the formula of percentage to volume. The volume of herbal medicine in one acid is 5mL in a concentration of 100% so that if it is included in the calculation it will produce as below :

$$V_1 \cdot \%_1 = V_2 \cdot \%_2$$

$$V_1 \cdot 100\% = 100\text{mL} \cdot 25\%$$

$$V_1 = 25\text{mL}$$

We get  $V_1$  in the calculation of 25mL so that the amount of herbal medicine used in dilution using distilled water is 25mL or as many as 5 packs of herbal medicine with a size of 5mL The addition was carried out until the solution had a volume of 100 mL which was carried out in a volumetric flask. The dilution process with a concentration of 12.5% was carried out in the same way. This dilution is carried out so that during the calculation process using UV-Vis the absorption value of herbal medicines against DPPH can be read. From the calculation of the absorption value of herbal medicines, the absorbance value is obtained as shown in the table below:



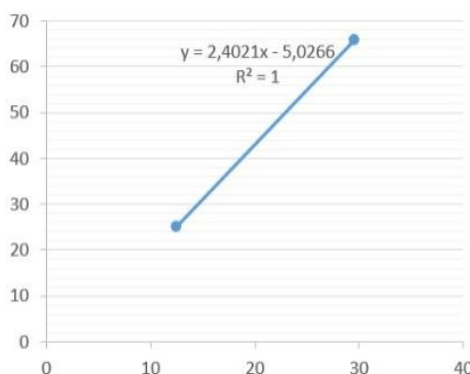


**Table 2.** Test results of antioxidant activity of herbal medicines against DPPH

Concentration (%)	Absorbance	(%) Inhibition
12,5	0,2123	29,50
25	0,4738	65,84

From the data table above, it is found that the percent inhibition value for each concentration has a different value for the inhibition value at a concentration of 12.5% is 29.50% and for a concentration of 25%, it has an inhibition value of 65.84%. So that the greatest inhibition value was obtained at a concentration of 25%. Percent inhibition was obtained from a difference between control absorbance sample [22]

From the results of the inhibition value, the IC<sub>50</sub> value of herbal medicine can be calculated. IC<sub>50</sub> is an indicator of antioxidant activity contained in an analyte, the smaller the IC<sub>50</sub> value, the greater the antioxidant activity. So that we get the form of graphs and regression values as follows :



**Figure 5.** Graph of antioxidant activity test results for Herbal Medicines

The IC<sub>50</sub> value of herbal medicine is obtained with a regression value of  $y=2.4021x - 5.0266$  so that the IC<sub>50</sub> value is 18.722 ppm and is still classified as having strong antioxidant content because the IC<sub>50</sub> value is less than 50 ppm. The best concentration of herbal medicine is shown at a concentration of 25% because it has a fairly large inhibition value of 65.84%, which means that this percentage value can be used as a large result of free radical reduction contained in DPPH solution with a concentration of 4 ppm. so that in the experimental test of the antioxidant activity of herbal medicines in combination with nanogold, a 25% concentration of herbal medicines was used.

**Nanogold Antioxidant Activity Test with DPPH**

Then the experiment was continued by calculating the presentation of the reduction of nanogold synthesis to DPPH. This experiment was carried inserting out by a solution of nanogold in the various concentrations of 5,10,15,20,25 and 30 in dark bottles which were then added with 4% DPPH solution with a ratio of nanogold and DPPH of 1:1. Testing of antioxidant activity on this research uses the DPPH method (1,1,2,2-diphenyl picryl hydroxyl). DPPH act as free radicals that can react with compounds that can donate a hydrogen atom [23]

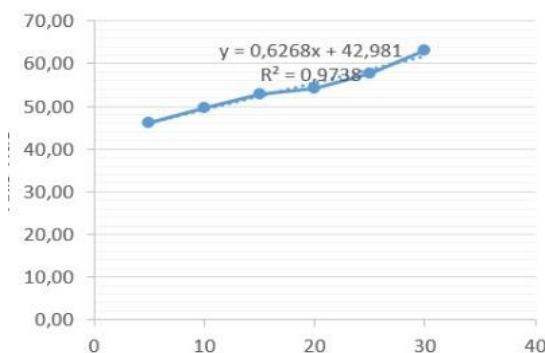
Furthermore, the nanogold that has been mixed with DPPH is left for 30 minutes so that the nanogold can react with DPPH and then measured using UV-Vis so that the data is obtained as shown in the following table:

**Table 3.** Nanogold Antioxidant Test Results against DPPH

Concentration (ppm)	Abs		% Inhibition
	Control	Nanogold	
5	0,8967	0,1202	46,14
10		0,161	49,68
15		0,2315	52,95
20		0,2990	54,23
25		0,3774	57,68
30		0,4191	63,02

From table 3. It can be concluded that the largest absorbance value is found in nanogold with the most concentrated concentration of 30 ppm at 0.4191 while the lowest absorbance value is shown at the smallest concentration of 5 ppm with an absorbance value of 0.1202.

From the table data above, when presented in graphical form, the antioxidant activity of nanogold in reducing DPPH radicals at various nanogold concentrations can be shown in Figure 6 below



**Figure 6.** % Free radical attenuation of DPPH against Nanogold

In the graph, it can be seen that there is an increase in the reduction of free radicals in DPPH along with an increase in the concentration of nanogold. The absorbance values of pure nanogold at concentrations of 5,10,15,20,25 and 30 ppm were 0.1202, 0.161, 0.2315, 0.2990, 0.3774, and 0.4191. The calculation results show the antioxidant activity of nanogold against DPPH which is indicated by a regression value of  $y = 0.6268x + 42.981$  and an  $IC_{50}$  value of 11.19 ppm.

$IC_{50}$  nanogold price < 50 so that an antioxidant compound is said to be a strong antioxidant. If the antioxidant compound with an  $IC_{50}$  value is less than 50 mg/L, it is said to be strong if the  $IC_{50}$  value is 50-100mg/L, being when the  $IC_{50}$  value is 100-150mg/L and is said to be weak when the price of  $IC_{50}$  is 150-200mg/L [24].

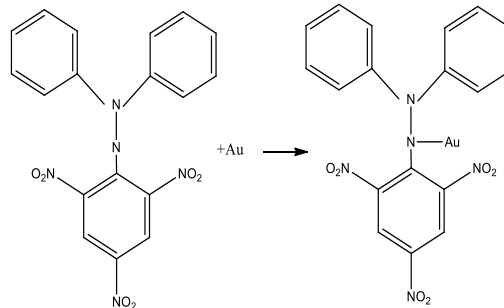
### Test the Effect of Adding Nanogold on Antioxidant Activity of Herbal Medicines

Then the experiment was continued by putting the herbal medicine in each dark bottle and then adding nanogold with a concentration of 5,10,15,20,25 and 30 ppm for each bottle with a ratio of 1:1, namely 3mL of 25% herbal medicine solution and 3mL nanogold solution at various concentrations. From the results of the addition of herbal medicines and nanogold, a yellowish solution was obtained for the addition of herbal medicines and nanogold with a concentration of 5ppm. The color change in the mixed solution of herbal medicine and nanogold occurred as the concentration of nanogold used increased.



**Figure 7.** Results of Adding Nanogold to herbal medicine

Then the DPPH. The solution was added 4% in the drug solution and nanogold in a 1:1:1 ratio, namely 3 ml of 25% herbal medicine solution, 3 ml of 4% DPPH solution, and 3 ml of nanogold solution, each bottle containing different concentrations of nanogold mixed solution is placed in a dark bottle. The use of placing it in dark bottles is because DPPH is sensitive to light so it can be easily oxidized.



**Figure 8.** The reaction of DPPH attenuation against nanogold

The more concentrated the concentration of nanogold added in herbal medicinal preparations made from haubatusauda, black garlic, and propolis, there will be an increase in the reduction of free radicals in the DPPH solution, so it can be concluded that the best concentration of nanogold used is the one with a high concentration. The determination of the highest antioxidant activity was indicated by a percentage of free radical scavenging in DPPH [10].

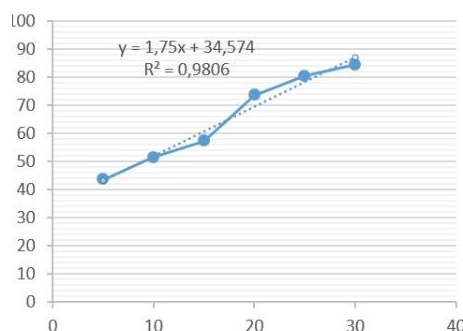
Based on the results of the calculation of the attenuation presentation, it is obtained that the concentration the most optimal in the process of reducing free radicals is at the highest concentration of 30 ppm with a large percent reduction of 84.42%. This shows that nanogold has high antioxidant properties and supports the process of reducing free radicals by using herbal medicines as the main ingredients that are safe for consumption. The following presents data on the results of the absorbance value of nanogold against DPPH

**Table 4.** Antioxidant test results of herbal and nanogold drugs against DPPH

Concentration (ppm)	Absorbance		% Inhibition
	Control	Nanogold + Herbal (25%)	
5	0,7042	0,5164	43,73
10		0,5018	51,60
15		0,5319	57,34
20		0,417	73,64
25		0,5151	80,44
30		0,5288	84,42

It can be seen from the table above that the absorbance value of nanogold mixed with herbal medicine with a concentration of 25% to DPPH is greater than the absorption value of pure herbal medicine. This is caused by an increase in antioxidant activity contained in herbal medicines and nanogold who collaborate.

From the table data above, when presented in graphical form, the antioxidant activity of nanogold with herbal medicine at various concentrations of nanogold is shown in Figure 9



**Figure 9.** % Free Radical Attenuation in DPPH for Herbal Medicine with The Addition of Nanogold





The graph shows an increase in free radical scavenging of DPPH along with the increase in the concentration of nanogold used. The absorbance values for each addition for concentrations of 5,10,15,20,25 and 30 ppm were 0,51, 0,50, 0,53, 0,41, 0,51, 0,52.

The calculation results show the antioxidant activity of herbal medicines and nanogold against DPPH as indicated by a regression value of  $y = 1.75x + 34,574$  and an  $IC_{50}$  value of 8.81 ppm. This shows that the antioxidant activity of the combination of herbal medicine and nanogold is quite strong.

The  $IC_{50}$  comparison between pure nanogold and nanogold and herbal medicine has a significant difference for the  $IC_{50}$  price of pure nanogold at 11.19 ppm while the  $IC_{50}$  price for the combination of nanogold and herbal medicine is 8.81 ppm. This shows that there is a decrease in the  $IC_{50}$  price in the combination of nanogold and herbal medicine so that it can be said that the antioxidant activity of the combination of herbal medicine and herbal medicine has decreased nanogold is stronger when compared to the antioxidant activity of pure nanogold so that nanogold can be used as a good combination in herbal medicine to maximize antioxidant activity.

## CONCLUSION

Based on the results research can conclude that :

1. The addition of nanogold concentration in herbal medicine can affect the antioxidant activity as indicated by the lower  $IC_{50}$  value
2. The best combination of herbal medicine and nanogold was obtained at the highest nanogold concentration, which was 30 ppm with a percentage of free radical reduction of 84.42%.

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