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Utilization of Nanogold and Nanosilver in Kelor (*Moringa Oleifera* Lam.) Leaf Extract for Pandemic of Covid-19

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ABSTRACT: This research has the purpose to determine the characteristics of the synthesis of 20 ppm nanogold using TEM, determine the effect of adding nanogold on the antioxidant activity of Moringa leaves, and determine the best concentration that supports increasing antioxidant activity in Moringa leaves. Nanogold and nanosilver were synthesized using bottom-up methods. The concentrations used for Moringa leaf extract and nanogold were 5, 10, 15, 20, 25, and 30 ppm. The concentration of the nanosilver used is 20 ppm. Nanosilver at a concentration of 20 ppm had the best inhibition of antibacterial activity. Testing of antioxidant activity was carried out using the DPPH method which was analyzed with a UV-Vis spectrophotometer. The results of the TEM nanogold test have a dominant cluster size of 22.17 nm. The best test results for the antioxidant activity of Moringa leaf extract at a concentration of 30 ppm was 60.7258 ppm. The addition of nanogold with a greater concentration of 30 ppm Moringa leaf extract resulted in greater antioxidant activity. The results showed that the best concentration of nanogold as a supporter of antioxidant activity in Moringa leaves was 30 ppm with a percent reduction of 79.288 ppm (very strong category).

KEYWORDS: Antioxidant Activity, Moringa Leaf, Nanogold, Nanosilver, Percent Reduction.

INTRODUCTION

The spread of the coronavirus (Covid-19) outbreak in Indonesia is increasing and worrying. The continuous increase in cases every day is due to the community's non-compliance with health protocols commonly referred to as the new healthy living habit by implementing 5M, namely wearing masks, washing hands with soap and running water, maintaining distance, staying away from crowds, and limiting mobilization and interactions. Non-compliance with health protocols that continue to be ignored has led to an increase in positive cases of covid in Indonesia. Conditions like this make people increasingly anxious and worried which has an impact on the decline in the body's immune system.

An immune system is a form of body defense in protecting itself from harmful foreign materials such as bacteria, fungi, parasites, and viruses. As is well known, if the human immune system is low, it will make it easier for viruses to attack and cause disease, including the Covid-19 virus. A breakthrough is needed to prevent contracting the Covid-19 virus, namely by increasing the body's immunity. One of them is the use of immunostimulants.

Immunostimulants are compounds that can stimulate the immune system and improve the function of the impaired immune system [1]. In times like today, many people have chosen to consume immunostimulants made from natural ingredients. An example of this natural ingredient is Moringa which has the Latin name Moringa oleifera Lam. The content in Moringa leaves includes flavonoids, phenols, alkaloids, and [1]. Flavonoids have a role as antioxidants and can stop free radical chain reactions, while saponins function as immunostimulant agents [2]. Moringa leaves will provide maximum benefits when combined with ingredients that support the antioxidant work of Moringa leaves themselves, namely nanogold and nanosilver.

Bioavailable nanogold helps the glutathione optimally by forming more effective clusters. Synergistic glutathione molecules in clusters formed with nanogold will increase the work of endogenous antioxidants. The synergistic power of antioxidants like this is very much needed during this pandemic season. Nanosilver antivirus can be used on Moringa leaves to support antivirus updates, especially for the coronavirus [3].

Nanogold and nanosilver are nanoparticles [4]. With such a small size, it will produce a more active cross-sectional area where the atoms interact because the surface of the material contains a greater number of atoms, making it easier for cells to enter and leave without disturbing the cell's working system [5]. In a study conducted by Ningtias (2021), nanogold-nanosilver packaged in healthy water became a material that can prevent exposure to the coronavirus in terms of increasing immunity in the body both

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in sick and healthy conditions. An increase in the body's immune system produces antibodies that function to fight any pathogens that enter the body.

Based on the existing background, this study aims to determine the antioxidant activity of antioxidant compounds contained in Moringa leaves and nanogold. Further testing was carried out on Moringa at the concentration with the highest antioxidant activity with nanogold.

MATERIALS AND METHODS

Tools and Materials

The equipment used includes an analytical balance, glass funnel, volumetric flask, beaker glass, measuring cup, dropper, hot plate, magnetic stirrer, micropipette, and UV-Vis Spectrophotometer. The materials used include Moringa leaves, 1000 ppm $HAuCl_4$ solution, 1000 ppm $AgNO_3$ solution, aquadest, sodium citrate, DPPH powder, and 96% ethanol.

Methods

Nanogold Synthesis

The synthesis of nanogold was carried out by the bottom-up method. Nanogold was made with various concentrations of 5, 10, 15, 20, 25, and 30 ppm. The first step is to take 100 mL of distilled water in 6 beakers heated to boiling. Then, 1000 ppm $HAuCl_4$ solution was added to 6 beakers each containing 3 mL of distilled water which was heated successively; 2.5 mL; 2 mL; 1.5 mL; 1 mL; and 0.5 mL. Addition of reducing agent sodium citrate as much as 0.3 grams in each beaker. Each solution is stirred and left until the previous solution is yellow (gold ions) to become colorless, turning dark blue, red, and finally wine red. Synthesis of nanogold at a concentration of 20 ppm was characterized by using TEM.

Nanosilver Synthesis

Synthesis of nanosilver was made at a concentration of 20 ppm. The first stage is heated as much as 100 mL of distilled water in a beaker. 2 mL of 1000 ppm $AgNO_3$ was added to the heated distilled water and left until a yellow color was formed in the solution.

Preparation Moringa Leaf Extract

Moringa leaves are converted into powder by being dried and exposed to indirect sunlight. After the Moringa leaves dry, they are smoothed and sifted 100 mess.

Preparation of Moringa Leaf Extract

Moringa leaves that have become powder are taken as much as 0.01 grams and dissolved in 100 mL of distilled water. Stir until all the powder is dissolved. Then, filtered to separate the filtrate and residue. The extract obtained was evaporated using a vacuum rotary evaporator with a given rotation speed of 60 rpm at a temperature of ± 40°C. Moringa leaf extract was obtained which was then diluted into 6 concentration variations are 5 ppm, 10 ppm 15 ppm, 20 ppm, 25 ppm, and 30 ppm.

Sample Preparation

Samples that were prepared include nanogold taken as much as 2 mL and put in 6 test tubes. Then, 0.04% DPPH solution was added with a ratio of 2: 1 so that 1 mL of DPPH solution was added. Samples were incubated for 30 minutes covered with aluminum foil.

The next sample of Moringa leaf extract was taken as much as 3 mL and put in 6 test tubes. 3 mL of nanogold was taken and put in 6 test tubes to which Moringa leaf extract had been added and then homogenized. Next, 0.04% DPPH solution was added in a 2:1 ratio so that 3 mL of DPPH solution was added to each test tube. The samples were incubated for 30 minutes with the test tubes covered with aluminum foil.

Antioxidant Activity Test

The incubated samples were tested on a UV-Vis spectrophotometer to obtain absorbance. Before testing the sample, measurements were made on the control, namely 96% ethanol. Next, measurements were taken to be the maximum wavelength (λ) of the DPPH solution. After that, the samples were tested at max DPPH.

The absorbance value of each sample is entered in the formula for % attenuation (%P) with the following equation:

$$\% P = \frac{Ab+As}{Ab} \times 100\%$$

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The results of the % reduction obtained are plotted with the concentration of each sample to obtain a curve with a regression equation that is useful in determining the IC_{50} value of nanogold and Moringa leaf extract with the addition of nanogold.

RESULTS AND DISCUSSION

Nanogold Synthesis

The synthesis of nanoparticles can be carried out by 2 methods, namely the physical method (top-down) and the chemical method (bottom-up) [6]. In this research, the method used is bottom-up. The synthesis of nanogold was carried out with 1000 ppm $HAuCl_4$ with the addition of sodium citrate as a reducing agent. It's in nanogold synthesis is needed as an electron donor (reduction agent) to convert gold metal ions (Au^{3+}) which can be called auric/aurat in the initial state of synthesis into gold metal ions that are not oxidized or have no charge (Au^0). Sodium citrate can function as a stabilizing agent for nanogold. Citrate ions that have a negative charge will work around the surface of the nanogold. This will make the nanogold not easy to aggregate. If a stabilizing agent is not given, the gold atoms will bond with each other, resulting in the formation of large nanogold clusters and nanogold aggregation. The nanogold synthesis process occurs as follows:

$$2Au^{3+} + 4C_6H_5O_7^{3-} + 6H_2O \rightarrow 2Au + 4C_6H_8O_7 + 3O_2$$

The synthesis of nanogold was carried out with variations in 5 concentrations including 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm. Heating is done until the color changes from clear yellow to wine red.

Figure 1. Nanogold with the smallest concentration from left to right

The color of the nanogold synthesis in **figure 1** was produced at the six variations in different concentrations. This is due to the difference in the number of clusters formed. At the highest concentration, the color produced is getting darker, which indicates that more and more nanoclusters are formed [7].

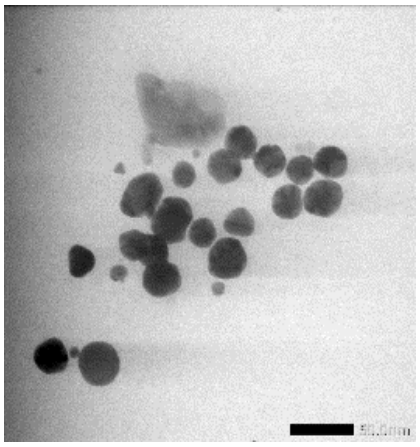

Figure 2. TEM analysis results in nanogold 20 ppm with a scale of 50.0 nm

Based on the results of the 20 ppm nanogold TEM test in **Figure 2**, shows that the shape of the nanogold cluster resembles an asymmetrical circle with various sizes. Based on the measurement results obtained, the dominant size of the nanogold cluster is 22.17 nm. Research by Taufikurohmah (2020), the dominant cluster size of nanogold is 20.68 and the size of nanogold that has been widely used is in the range of 1-100 nm [8].

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Nanosilver Synthesis

The nanosilver synthesis used the same method as the nanogold synthesis, bottom-up. Synthesis of nanosilver was carried out at a concentration of 20 ppm by chemical reduction. Sodium citrate was used as a reducing agent for $AgNO_3$.

In nanosilver synthesis, sodium citrate will serve to provide citrate ions which will form complexes with silver ions [8]. Silver ions with a charge of +1 (Ag^+) will be reduced to nanosilver which has no charge (Ag^0). The reactions that occur in the synthesis of nanosilver are as follows:

$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2\uparrow$

The formed nanosilver was characterized by a color change from colorless $AgNO_3$ to yellow. **Figure 3** is the result of the synthesis of nanosilver at a concentration of 20 ppm.

Figure 3. Nanosilver 20 ppm

Nanosilver at a concentration of 20 ppm had the best inhibition on antibacterial activity with the largest clear zone diameter of 19.38 mm which was included in the category of strong bacterial inhibitory response [9]. The ability of nanosilver as an antibacterial can be used to kill various types of bacteria to viruses that cause disease in humans.

Preparation of Moringa Leaf Extract

Moringa leaf extract was obtained by the maceration method. This method will dissolve the active compound in Moringa leaves. This can happen because there are different concentrations between the inside and outside of the active compound solution [11]. Powder of Moringa leaves which has a function surface area becomes larger. The larger the surface area of the Moringa leaves, it will provide maximum results during the extraction process. The active antioxidant compounds contained in Moringa leaves will be more extracted.

Extraction of Moringa leaves used a solvent in the form of aquadest. This solvent is polar which can dissolve some secondary metabolites. Aquadest solvent is used in this extraction because it is guaranteed that it will not be harmful if the results of the extraction are applied to food products consumed by humans.

The extract obtained in the maceration process was evaporated using a vacuum rotary evaporator with a rotation speed of 60 rpm at a temperature of ± 40°C. The result obtained is a thick extract from Moringa leaves. Then, the thick extract was dissolved using aquadest 6 concentration variations**.**

Moringa Leaf Extract Antioxidant Activity Test

Moringa leaf extract which has been varied in concentration was tested for antioxidant activity with radical absorption by DPPH (1.1-diphenyl-2-picrylhydrazyl). Free radicals in DPPH will capture hydrogen compounds contained in antioxidant compounds. The instrument used in the antioxidant test with DPPH is a UV-Vis spectrophotometer.

The absorbance value will be obtained when free radicals in DPPH interact with antioxidant compounds marked by a change in DPPH color from dark purple to yellowish. Before carrying out antioxidant testing on samples, the maximum wavelength was measured for 0.04% DPPH in the wavelength range of 400-800 nm. The results show that the maximum wavelength of 0.04% DPPH is 517 nm and the absorbance value is 0.8652. This maximum wavelength of DPPH will be used in the measurement of Moringa leaf extract samples and the synthesis of nanogold. The absorbance value obtained will be used in determining the attenuation of DPPH free radicals.

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Moringa leaf extract with various concentrations was tested for antioxidant activity by preparing each sample of Moringa leaf extract with various concentrations to be mixed with DPPH. The comparison between the sample and DPPH is 2:1. Moringa leaf extract sample was 3 mL and 1.5 mL of 0.04% DPPH solution. Each sample was put in a dark bottle and incubated for 30 minutes. The use of this dark bottle aims to prevent damage to the DPPH solution which will affect the absorbance results. After incubation, measurements were made on the six prepared samples. The results of the absorbance values are presented in the form of **table 1**.

Table 1. Moringa leaf extract antioxidant activity test results

Concentration (ppm)	Absorbance	% Reduction
5	0.6292	27.2769
10	0.6125	29.2071
15	0.5234	39.5053
20	0.4739	45.2265
25	0.4042	53.2824
30	0.3398	60.7258

Percent reduction is the ability of an active compound to slow down the activity of free radicals. The percentage of reduction that has been obtained will be correlated with the concentration of the extract into a regression curve to obtain a linear regression equation. This equation will be used in determining the IC_{50}. It indicates the amount of concentration of an antioxidant compound in reducing 50% of free radicals [12]. The results of the regression curve are presented in **Figure 4**.

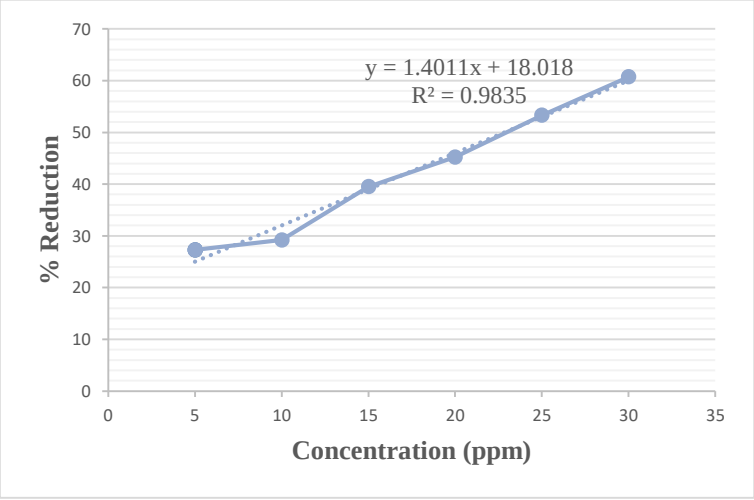

Figure 4. Moringa extract antioxidant activity

Based on the curve, the regression equation is y = 1.4011x + 18.018. With this equation, the IC_{50} value of Moringa leaf extract is 22.826 ppm. IC_{50} value <50 ppm belongs to the category of the intensity of very strong antioxidant ability.

Moringa Leaf Extract Antioxidant Activity Test with Addition of Nanogold

The results of the synthesis of nanogold with six concentration variations were first measured for nanogold uptake and against DPPH free radicals. Nanogold was measured without DPPH because it has its absorption. Measurements were made at the maximum wavelength of the DPPH, which is 517 nm.

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Tests were carried out on each concentration of nanogold with a ratio of 2:1 DPPH. Then, it was incubated for 30 minutes and the absorbance value of the mixture of nanogold and DPPH with the absorbance value of nanogold. It was found that the percentage of reduction increased with the increasing concentration of nanogold. The results can be seen in **table 2**.

Table 2. Nanogold antioxidant activity test results

Concentration (ppm)	Abs. Nanogold (NG)	Abs. NG+DPPH	Sample abs.	% Reduction
5	0.1202	0.6045	0.4843	44.0245
10	0.1613	0.6087	0.4474	48.2894
15	0.2318	0.6604	0.4286	50.4623
20	0.2961	0.7037	0.4076	52.8895
25	0.377	0.7534	0.3764	56.4956
30	0.4189	0.7868	0.3679	57.478

Free radicals in DPPH namely N atoms will interact with nanogold on Au atoms. The bond that occurs between the two is a coordinating covalent bond. Au atoms will capture free radicals, namely N atoms so that Au-N bonds will form. With the formation of these bonds, the N atom will be stable. This shows that the Au atom in nanogold can reduce the free radicals contained in DPPH. The reaction that occurs is shown in **Figure 5.**

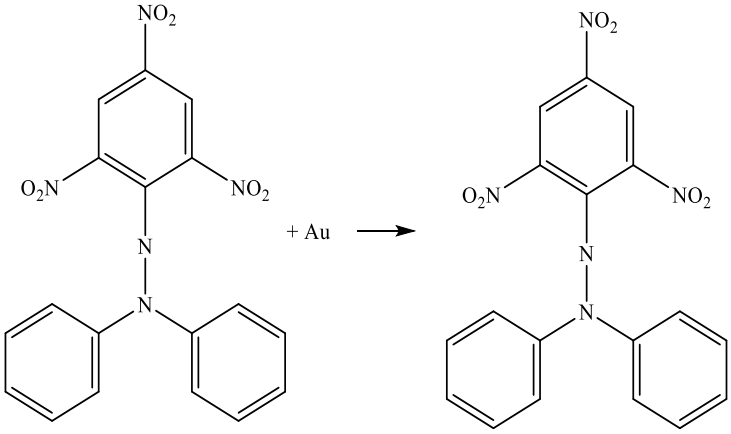

Figure 5. Reduction reaction between nanogold and DPPH

Au atoms will capture free radicals, namely N atoms so that Au-N bonds will form. With the formation of these bonds, the N atom will be stable. This shows that the Au atom in nanogold can reduce the free radicals contained in DPPH. The reaction that occurs is shown in **Figure 5.**

The linear regression equation in **figure 6** is y = 0.5389x + 42.175 and the IC_{50} is 14.52 ppm. The IC_{50} result of nanogold is a very strong category of antioxidant activity intensity.

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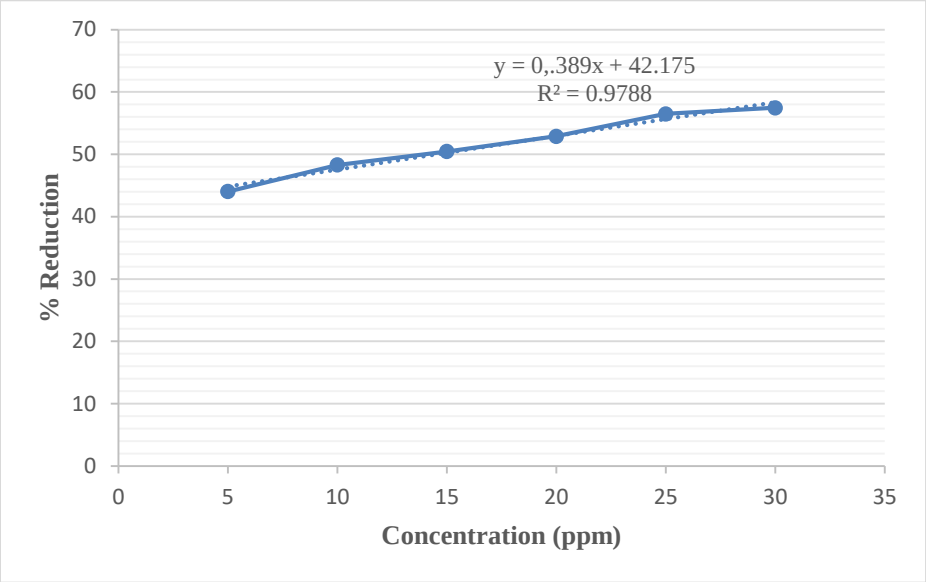

Figure 6. Nanogold antioxidant activity curve

Subsequent tests were carried out on Moringa leaf extract with a concentration of 30 ppm. Moringa was combined with nanogold at all concentration variations. Measurement of antioxidant activity was carried out at the same wavelength, namely 517 nm. The volume ratio used is 1:1:1, with a volume of 1.5 mL at all. The sample was incubated for 30 minutes, then the absorbance was measured. The results obtained are presented in **table 3**.

Table 3. The results of reducing Moringa leaf extract with the addition of nanogold

Concentration (ppm)	Abs. Nanogold (NG)	Moringa Leaf extract	Sample abs.	% Reduction
5	0.1202	0.6599	0.5397	37.6213
10	0.1613	0.6397	0.4784	44.7064
15	0.2318	0.6278	0.396	54.2302
20	0.2961	0.6072	0.3111	64.043
25	0.377	0.5993	0.2223	74.3065
30	0.4189	0.5981	0.1792	79.2880

This shows that the greater the concentration of nanogold given, the greater the ability of colloids in nanogold to reduce free radicals. The results of the linear regression equation curve are presented in **figure 7**.

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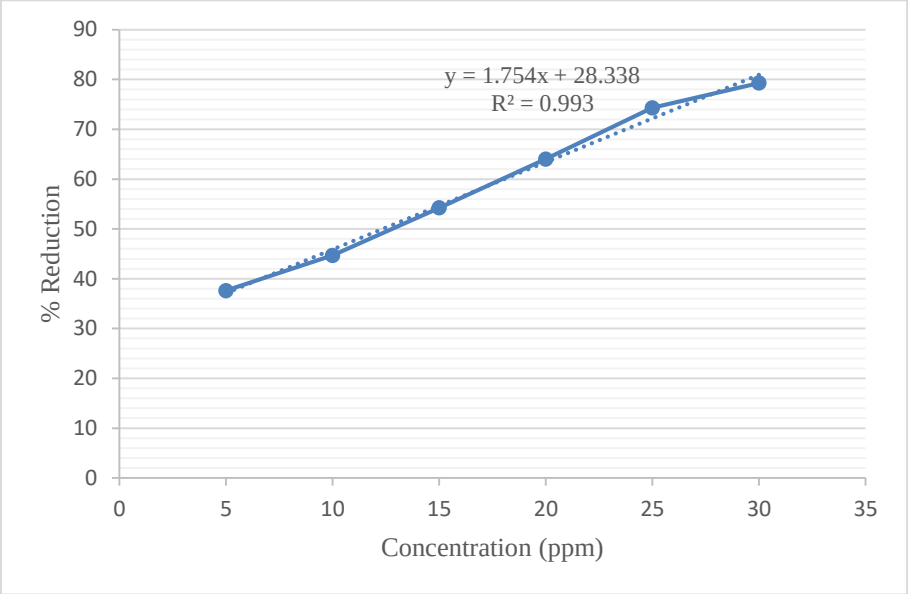

Figure 7. Moringa leaf extract antioxidant activity curve with the addition of nanogold

The results of the regression curve show the equation y = 1.754x + 28.338 and IC_{50} is 12.35 ppm. The IC_{50} result in the mixture of moringa with nanogold is a very strong category of antioxidant activity intensity.

Table 4. Percent reduction of all samples at the same concentration

Concentration (ppm)	Percent Reduction			
	Moringa Leaf Extract	Nanogold (NG)	Moringa leaf extract + NG	combining Moringa leaf extract with nanogold
2.5	13.6384	22.0125	35.6509	37.6213
5	14.6035	24.1447	38.7482	44.7064
7.5	19.7536	25.2311	44.9847	54.2302
10	22.6132	26.4447	49.0579	64.043
12.5	26.6412	28.2478	54.889	74.3065
15	30.3629	28.739	59.1019	79.2880

This shows that the greater the concentration of nanogold given, the greater the ability of colloids in nanogold to reduce free radicals. The results of the linear regression equation curve are presented in **figure 8**.

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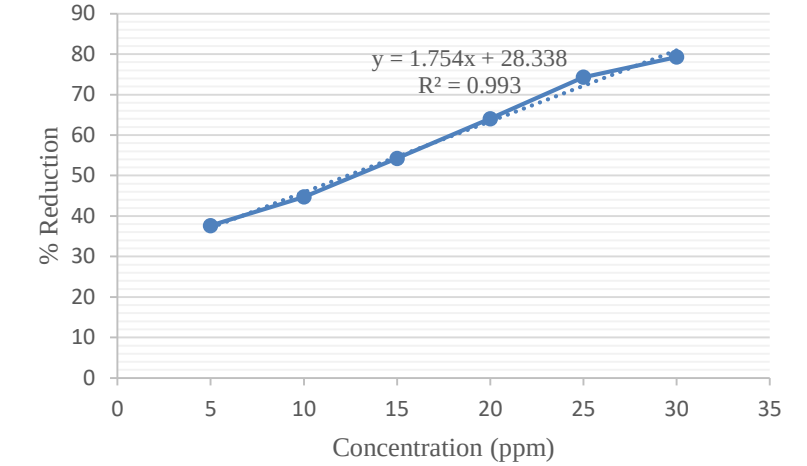

Figure 8. Moringa leaf extract antioxidant activity curve with the addition of nanogold

The results of the regression curve show the equation y = 1.754x + 28.338 and IC_{50} is 12.35 ppm. The IC_{50} result in the mixture of moringa with nanogold is a very strong category of antioxidant activity intensity.

Based on the results in **table 4**, it can be seen that %P of combining Moringa leaf extract and nanogold has the highest results with the same concentration in all samples. This can happen because there is a synergistic combination of Moringa leaf extract and nanogold on the effect of reducing free radicals also known as the synergistic effect of antioxidants.

Moringa leaf which has antioxidant properties will match with nanogold which has antioxidant properties as well, which can be proven by the synergism in the results of the percent reduction that has been tested. Thus, with the results of very strong antioxidant activity, the incorporation of nanoparticle technology, especially nanogold and nanosilver with natural ingredients from Moringa leaves, is vertay potential if used in maintaining the body's immune system, especially during the Covid-19 pandemic.

CONCLUSIONS

Nanogold and Moringa leaf have very strong antioxidant activity at a concentration of 30 ppm. The addition of nanogold with increasing concentration in Moringa leaf resulted in greater intensity of antioxidant activity with an IC_{50} 12.35 ppm.

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