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ANTIOXIDANT ACTIVITY TEST OF A FUNCTIONAL DRINK MIXED FROM MORINGA LEAVES DECOCTION EXTRACT (Moringa oleifera) AND GARLIC DECOCTION EXTRACT (Allium sativum) USING THE DPPH METHOD

Youstiana Dwi Rusita [1*], Rini Tri Hastuti [1],
[1]Department of Pharmaceutical and Food Analysis, Health Polytechnic, Ministry of Health, Surakarta,
Jl. Ksatrian No.2 Danguran, South Klaten 57425, Klaten, Central Java, Indonesia
*e-mail: ratihpurwasih0509@gmail.com

ABSTRACT

Antioxidants are essential to increase endurance and maintain the immune system to prevent the entry of various diseases. Antioxidants are found in many fruits and vegetables, one of which is moringa and garlic. Moringa leaves and garlic have properties as a source of antioxidants and can increase the body's immune system so that it can be processed into functional drinks. The manufacture of functional drinks MOSA (Moringa oleifera (Leaves) & Allium sativum (Garlic)) uses the flavor of palm sugar, which is known to have antioxidant activity. The purpose of this study was to determine the pH and antioxidant activity of functional beverages MOSA. In this study, pH testing used a pH meter, and antioxidant activity testing used a UV-Vis spectrophotometer with the DPPH method. The maximum wavelength of the control solution obtained is 518 nm, with an absorbance of 0.786. The results showed that the average pH was 6.574, and the IC_{50} was 45.931. The low pH in MOSA functional drinks is low acidic and has very strong antioxidant activity. The low pH value of MOSA functional beverage meets the quality requirements after MOSA is dissolved with water so that it has the potential to be a functional drink.

Keywords: moringa leaves, garlic, antioxidant, functional drink, DPPH method

INTRODUCTION

During a pandemic, people are required to maintain their immune systems so that viruses do not easily attack them. The immune system can be increased by consuming nutritious foods and sources of bioactive components that have antiviral and immunomodulatory/immune activity boosters. Consumption of foods rich in vitamins, minerals, and bioactive ingredients can be in the form of staple foods, various vegetables and fruits, animal products, and functional foods and drinks from herbs (Dixit *et al*., 2023).

Plants are one of the oldest natural ingredients known as a source of functional food. Functional food can be food and drinks derived from animals or plants (Yuniastuti, 2014). The body needs antioxidants to neutralize free radicals that can help protect the body from free radical attacks and reduce their negative impacts (Wahid *et al*., 2017).

The making of functional drinks based on moringa leaves is done because it is known that moringa leaves have antioxidant compounds that are beneficial for body health. As in the research of Verma *et al*., 2009, that moringa leaves contain a large amount of phenol which is known as a free radical scavenger. In rural areas, consumption of moringa leaves is limited to processed vegetables in soup such as clear vegetables and fresh vegetables (Rahmawati, Putri Safrida & Adi, 2016). The addition of garlic to this moringa leaf drink can be useful for increasing the efficacy of the drink, because it is known from the research results of Prasonto *et al.*, 2017 that Garlic (Allium sativum) has antioxidant activity. Research by Rizkayanti *et* *al*. (2017) shows that fresh moringa leaf water extract has a strong antioxidant activity of 57.54 ppm. Research by Azhar *et al*. (2021) shows that garlic ethanol extract has a very strong antioxidant activity of 28.524 ppm.

Based on the background above, the author is interested in conducting research on antioxidant activity test of a functional drink preparation mixed from moringa leaf aquades extract and garlic aquades extract using the DPPH method. Thus, it is expected to produce a beverage that contains high antioxidant activity.

METHODS

The research design used is descriptive because it describes the method of making and testing the antioxidant activity of MOSA functional drinks. The research variable is a single variable, namely the antioxidant activity test of the functional drink MOSA.

Materials

Fresh moringa leaves, garlic, palm sugar, water, 1-1 diphenyl-2- picrylhydrazyl (DPPH), methanol PA, aluminum foil and distilled water.

Tools

Pot, stove, sieve, knife, scale, pH meter (*Capp*), measuring flask (*Iwaki*), test tube, micropipette (*Capp*), beaker (*Iwaki*), analytical balance (*Labex*), measuring cylinder (*Iwaki*), label, and UV-VIS spectrophotometer (*Raptor*).

The Course of Research

This stage includes the method of making, organoleptic test, pH test and antioxidant activity test of MOSA functional drinks

Manufacture of MOSA functional drinks

Table 1. MOSA Functional Drink Formula

Material	Formula
Moringa leaves	5 g
Garlic	2 g
Palm sugar	50 mL
Water	600 mL

Extraction of moringa leaves and garlic with decoction method

Moringa leaves that are still fresh, soak and wash and then drain, if the water is dry, then weigh 5 g. A total of 2 g of garlic is washed and then crushed. Moringa leaves and garlic are put in a pot and then pour 600 mL of water, heat on the stove over low heat until boiling and leave for 10 minutes. Then it is filtered and put in a glass. Then, add 50 mL of dissolved palm sugar (Modified from Affandi, 2019).

Organoleptic test

How to test samples using human senses. With elements of aroma, taste, and color that are part of organoleptics (Suryono *et al*., 2018).

pH test

The pH value measurement was carried out using a pH meter that had been calibrated using a pH 4 and pH 7 buffer solution. pH measurements were carried out three times (Anggraini, 2014).

Antioxidant activity test by DPPH method

1) **Manufacture of DPPH solution (50 ppm)**

   1,1-Diphenl-2-picrylhydrazyl (DPPH) is weighed as much as 5 mg then dissolved with PA methanol and put into a 100 mL measuring flask that has been coated with aluminum foil, add methanol to the limit mark (Nadhiroh, 2018).

2) The making of control solution

A total of 1 mL of DPPH 50 ppm solution is added with 2 mL of methanol. The mixture is homogenized and incubated in room temperature, away from the sun for 30 minutes (Tristantini et al., 2016).

3) Determination of optimal wavelength

The determination of the maximum wavelength of the DPPH solution was carried out using a control solution and its absorption was measured in the wavelength range of 510 – 520 nm with methanol blanks (Yuliani & Dienina, 2015).

4) Absorbance measurement

The test solution from 5 series concentrations of 5, 6, 7, 8, 9 ppm was taken 2 mL and put in a test tube, then 2 mL of DPPH reagent solution was added. Homogenized and incubated for 30 minutes, then measured for absorption at maximum wavelength (Nadhiroh, 2018).

5) Data analysis

The analysis of the antioxidant testing of the DPPH method was carried out by looking at the results of absorbance measurements obtained using a UV-VIS spectrophotometer. Furthermore, the percentage of free radical reduction of DPPH is calculated using the formula:

$$\% \text{ Inhibition} = \frac{Control\ Abs - Sample\ Abs}{Control\ Abs} \times 100\%$$

Information :
% Inhibition : percentage of DPPH free radical capture
Control abs : absorbance of DPPH with methanol
Sample abs : absorbance of the sample after being reacted with DPPH

The antioxidant activity power of MOSA functional beverage was then calculated with the IC_{50} price through linear regression obtained from the sample concentration graph compared to the percent attenuation. The formula for the linear regression equation is as follows:

$$y = a + bx$$

Information :
y = percent inhibition with a value of 50%
x = sample concentration
a = intercept
b = regression coefficient

Then the results of the linear regression analysis in the form of a value of x, are included in the formula IC_{50} = antilog x and the level of antioxidant strength is determined.

Table 2. Levels of Antioxidant Strength

Intensity	IC_{50}
Very powerful	< 50 µg/mL
Strong	50-100 µg/mL
Keep	100-150 µg/mL
Weak	150-200 µg/mL
Inactive	> 200 µg/mL

(Source : Molyneux, 2004 in Yuliani & Dienina, 2015)

RESULT AND DISCUSSION

MOSA Functional Drink Formula (*Moringa oleifera* (Moringa leaf) & *Allium sativum* (Garlic))

Modification of the composition of the MOSA drink formula needs to be done to get the best results. The composition used is 5 g of light green fresh moringa leaves, 2 g of garlic, 600 mL of water, and 50 mL of palm sugar. When using an unmodified formula, a less sweet drink was obtained and the smell of garlic was still very strong. Furthermore, modifications were made to the composition of MOSA functional beverage ingredients. In this study, the MOSA functional drink formula has been modified by changing the composition of ingredients. The MOSA functional drink formula used is 5 g of light green fresh moringa leaves, 2 g of garlic, 600 mL of water, and 50 mL of palm sugar. Modifications are made to get good results, because before the modification is made, a less sweet drink is obtained and the smell of garlic is still very smelly.

Organoleptic Test of MOSA Functional Drinks

This test was carried out using the human senses, with aroma, taste, and color elements in MOSA functional drinks. The results of the MOSA functional beverage organoleptic test are shown in Table 3.

Table 3. MOSA Functional Beverage Organoleptic Test Results

Kind	Aroma	Taste	Color
MOSA Functional Drink (*Moringa oleifera (Moringa* leaf) & *Allium sativum* (Garlic)	Typical of moringa leaves and garlic	Sweet palm sugar	Yellowish brown

Organoleptic testing on MOSA functional drinks has a distinctive aroma of moringa leaves and garlic, tastes sweet, and has a Yellowish brown color. Organoleptic testing of MOSA functional drinks is by observing aroma, taste, and color. MOSA functional drink has a distinctive aroma of moringa leaves and garlic, tastes sweet palm sugar, and is yellowish-brown in color. This yellowish-brown color is caused by the addition of palm sugar. As stated in Susi (2013) that palm sugar has a yellowish brown color.

pH Test of MOSA Functional Drinks

The results of the pH test of MOSA functional beverages using a pH meter are shown in Table 4.

Table 4. Results of the MOSA Functional Beverage pH Test

Kind	Replication		
	I	II	III
MOSA Functional Drink (*Moringa oleifera (Moringa* leaf) & *Allium sativum* (Garlic)	6,572	6,576	6,576
Average		**6,574**	
SD		**0,002**	

This test was carried out to determine the pH value of MOSA functional drinks using a pH meter calibrated with pH 4 and pH 7 buffers. The pH test begins by calibrating the pH meter using a pH 4 then pH 7 buffer solution, which aims to ensure that the measurement results from the instrument are acceptable and within the required validation range (Yusuf, *et al.*, 2018). The results of the MOSA functional beverage pH test were obtained on average, which was 6.575. The pH of MOSA functional drinks is said to have acidic properties because it has a pH between 0 and 7. As stated by Karangan, *et al* (2019) that acidic properties have a pH between 0 to 7 and alkaline properties have a pH value of 7 to 14. The pH of MOSA functional drinks is included in the low-acidic group. As stated by Yulianti (2008) that low-acid foods are foods that have a pH above 5.3. The pH value is one of the important parameters to measure because it is related to the quality of a food product. Changes in pH value can change the taste and affect the shelf life of a food product (Anggraini, 2014). The low pH value of MOSA functional beverage meets the quality requirements after MOSA is dissolved with water so that it has the potential to be a functional drink (Adhayanti & Ahmad, 2019).

Test for Antioxidant Activity of MOSA Functional Drinks

The results of the measurement of % Inhibition of MOSA functional beverages using the DPPH (*1,1-Diphenil-2-picrylhydrazyl*) method are shown in Table 5. The DPPH method is one of the quantitative test methods to determine the activity power of MOSA functional drinks as an antioxidant. This method was chosen because it is a simple, easy, fast, and sensitive method. The test begins with the determination of the maximum wavelength of absorption or absorption of DPPH using a control solution. The maximum wavelength is the wavelength with the highest or maximum absorbance. The determination of the maximum wavelength aims to determine the wavelength of the compound to be measured to provide the most optimal absorbance (Sari, 2019). According to Yuliani & Dienina (2015) DPPH solutions have a wavelength range that ranges from 510 – 520 nm. In this study, the maximum wavelength of DPPH solution was obtained of 518 nm with an absorbance of 0.786. As in the research of Luthfiyani *et al.*, (2020) that the maximum wavelength of the DPPH solution obtained is 518 nm. Similar to the research of Suwarni & Cahyadi (2016), it is known that the maximum wavelength of DPPH is 518 nm. The wavelength obtained will be used to find the absorbance of samples that have been mixed with DPPH solution to determine their antioxidant activity.

Tabel 5. Results of % Inhibition Measurement of MOSA Functional Beverages with Maximum Wavelength (518 nm)

Concentration (ppm)	% Inhibition Absorbance					Linear Regression	IC_{50}
	R1	R2	R3	$\bar{X}$ %	SD		
5	2,671	2,671	2,671	2,671	0	y = 1,056x +	45,931
6	3.816	3,435	3,435	3,562	0,220	1,4964	
7	4,198	4,580	4,707	4,495	0,265	R^2 = 0,9952	
8	5,979	5,597	5,597	5,724	0,221		
9	6,488	6,870	7,252	6,870	0,382		

The result of the average percent of inhibition obtained, can be used to determine the linear regression equation. The linear regression equation obtained is y = 1.056x + 1.4964, this linear regression is used to determine IC_{50}. In this test, several concentrations were made from samples, namely 5 ppm, 6 ppm, 7 ppm, 8 ppm, and 9 ppm. Each concentration is added with DPPH solution, then incubated for 30 minutes. Incubation aims to bind substances that are antioxidants to DPPH radicals, then after incubation an absorbance reading with a wavelength of 518 nm is carried out. In each concentration, the average percentage of inhibition was obtained sequentially of 2.671; 3,562; 4,495; 5,724; and 6.87. The results of this study are in accordance with Sari (2019) research that with an increase in sample concentration, the absorbance of the sample will decrease and the value of the inhibition rate will increase. The average percent inhibition of each concentration is used to determine the linear regression equation. The result of the linear regression equation obtained is y = 1.056x + 1.4964, the result of this linear regression is used to determine IC_{50}. IC_{50} (*Inhibition Concentration* 50%) is a number that shows the concentration of samples that are able to inhibit the DPPH oxidation process by 50% (Yuliani & Dienina, 2015).

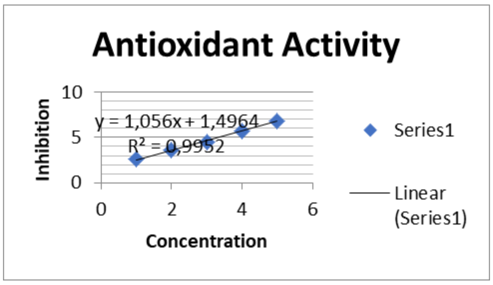

Figure 1. Curve % of antioxidant activity inhibition

After getting the linear regression equation, then the IC_{50} is calculated. From the calculation of the linear regression equation, an IC_{50} result of 45.931 was obtained, which means that MOSA functional drinks are included in the category of very strong antioxidants. According to Yuliani & Dienina (2015), the smaller the IC_{50} means the higher the antioxidant activity and specifically an antioxidant compound is said to be very strong if the IC_{50} value is less than 50 ppm. Moringa leaves contain alkaloids, saponins, phytosterols, tannins, phenolics, polyphenols and flavonoids. Moringa leaves also contain vitamin C. These compounds can prevent LDL-ox (Alverina *et al*., 2016). Moringa leaves also contain potassium as a blood pressure controller and phytospherols as a preventative against an increase in bad cholesterol in the blood (Yanti & Nofia, 2019). Garlic has the largest content of antioxidant compounds, namely *allicin* and *alliin* as anticholesterol and reduces the risk of heart disease. *Alliin* helps increase levels of HDL cholesterol or good cholesterol and slows down the synthesis of endogenous cholesterol. Research by Silagy and Neil in 1994 stated that garlic is a pioneer in fat loss (Setiawan *et al*., 2019).

CONCLUSION

Based on the research results, the following conclusions were obtained:

1. The MOSA functional drink formula used is 5 g, 2 g of garlic, 600 mL of water, and 50 mL of palm sugar;
2. MOSA functional drink has a distinctive aroma of moringa leaves and garlic, tastes sweet palm sugar, and is yellowish-brown in color;
3. The pH test results of MOSA functional drinks were obtained on average, which was 6.575. The pH of MOSA functional drinks is said to have acidic properties and belongs to the low-acidic group;
4. The results of the antioxidant activity test of MOSA functional drinks have an IC_{50} of 45.931 which means that it is included in the category of very strong antioxidants.

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