Jurnal Aisyah: Jurnal Ilmu Kesehatan

Volume 7, Issue 3, September 2022, p. 675–682
ISSN 2502-4825 (print), ISSN 2502-9495 (online)

Antioxidant Activity Test of "*Aia Tempayang*" With DPPH Metode (*2,2-Diphenyl-1 Picrylhydrazyl*)

Dera Elva Junita[1], Gemala Anjani[2*], Muflihatul Muniroh[3], Nurmasari Widyastuti[4], Anang M. Legowo[5], Ahmad Syauqy[6]

[1,2,4,6] Department of Nutrition Science Faculty of Medicine, Diponegoro University
[3] Department of Physiology, Faculty of Medicine, Diponegoro University
[5] Faculty of Food Technology, Diponegoro Univerversity

ARTICLE INFO

Article history:

Received 11 April 2022
Accepted 21 July 2022
Published 10 September 2022

Keyword:

Aia tempayang
Antioxidant
Sappan wood
Pangdahai
Basil seeds

A B S T R A C T

Aia Tempayang is a traditional West Sumatera drink made from sappan wood (caesalpania sappan), pangdahai/scaphium macropadum, and basil seeds (ocimum basilicum). Aia tempayang is simple to find and can be found in stalls that sell it in packs. The local society consumes aia tempayang because it is thought to reduce fever and treat canker sores. Sappan wood, pangdahai, and basil have antioxidant, anti-inflammatory, anticancer, and antibacterial properties. Because there has been no research on the health benefits of aia tempayang drink, the goal of this study was to determine the antioxidant activity of aia tempayang drink as well as the antioxidant activity of each ingredient. The DPPH method was used in this study to assess antioxidant activity (2,2-diphenyl-1). -Picrylhydrazil. aia tempayang drink by steeping dry sappan wood, pangdahai, and basil seeds in warm water for 20 minutes at 70^0Celsius, then filtering it. As a comparison, aia tempayang wrought material dissolved in ethanol was used. Comparison of components aia tempayang drink S: 56%, KS:9%, BS:35% made in 3 graded doses where dose 1 (S 4 g: KS 0.67 g: BS 2.5 g); dose 2 (S 8 g): KS 1.34 g: BS 5 g); and dose 3 (S 8 g): KS 1.34 g: BS 5 g) (S 16 g: KS 2.68 g: BS 10 g). The results of testing the antioxidant activity of aia tempayang drink showed an IC_{50} value of 4,417 g/l, while aia tempayang material in ethanol obtained an IC_{50} value of 2,018 g/ll. This shows that at a concentration of 4,417 g/l it can reduce free radicals by 50% where this result is the same as being located between dose 3 and dose 4. Testing the antioxidant activity of each ingredient according to the dose brewed with warm water obtained IC_{50} values as follows: 0,852 g/l, pangdahai 0,480 g/l and basil seeds 2,197 g/l. This shows that pangdahai has a high activity compared to sappan wood and basil seed.

Kata kunci:

Aia tempayang
Antioksidan
Kayu secang
Kembang semangkuk
Biji selasih

**) corresponding author*

Gemala Anjani

Department of Nutrition Science Faculty of Medicine, Diponegoro University
Jl. Prof H. Soedarto, SH, Tembalang, 50275, Semarang, Indonesia

A B S T R A K

Aia Tempayang merupakan minuman tradisional khas Sumatera Barat yang berasal dari kombinasi kayu secang (*caesalpania sappan*), kembang semangkuk (*pangdahai/scaphium macropadum*) dan biji selasih (*ocimum basilicum*). *Aia tempayang* secara komersial dijual dalam 1 kemasan. Bagi masyarakat sekitar, *aia tempayang* dikonsumsi karena dipercaya dapat menurunkan panas dan mengobati sariawan. Tujuan penelitian ini adalah untuk mengetahui aktivitas antioksidan minuman *aia tempayang* serta aktivitas antioksidan masing–masing bahan. Metode pengujian aktivitas antioksidan yang digunakan dalam penelitian ini adalah metode DPPH (*2,2-difenil-1-pikrilhidrazil*). Persiapan minuman *aia tempayang* dilakukan dengan cara bahan kayu secang kering, kembang semangkuk, dan biji selasih diseduh dengan air hangat suhu 70^0C selama 20 menit, lalu disaring.

Email: gemaanjani@gmail.com
DOI: 10.30604/jika.v7i3.1023

Bahan kering *aia tempayang* yang dilarutkan dengan pelarut etanol digunakan sebagai pembanding. Perbandingan komponen *aia tempayang* yaitu S : 56% , KS : 9% , BS : 35% dibuat dalam 3 dosis bertingkat dimana dosis 1 (S 4 g: KS 0,67 g : BS 2,5 g) ; dosis 2 (S 8 g: KS 1,34 g : BS 5 g) dan dosis 3 (S 16 g: KS 2,68 g :BS 10 g). Hasil pengujian aktivitas antioksidan minuman *aia tempayang* menunjukkan niai IC_{50} 4,417 g/l , sedangkan bahan *aia tempayang* yang dilarutkan etanol diperoleh nilai IC_{50} sebesar 2,018 g/l. Hal ini menunjukkan pada konsentrasi 4,417 g/l dapat meredam radikal bebas sebanyak 50% dimana hasil ini sama dengan terletak diantara dosis 3 dan dosis 4.Pengujian aktivitas antioksidan masing – masing bahan sesuai dosis yang diseduh dengan air hangat diperoleh nilai IC_{50} sebagai berikut kayu secang 0,852 g/l, kembang semangkuk 0,480 g/l, dan biji selasih 2,197 g/l. Hal ini menunjukkan kembang semangkuk memiliki aktivitas yang tinggi dibandingkan kayu secang dan biji selasih.

INTRODUCTION

The current pattern of human life has changed over time. The diet is one example of a life pattern that has changed dramatically. An unhealthy diet combined with exposure to harmful substances in the body can lead to degenerative diseases and conditions, especially in light of the current situation with the Corona virus – 19, which necessitates the maintenance of one's immune system. Most diseases are caused by excessive oxidation reactions in the human body's cells. In small amounts, free radicals are beneficial to health, such as fighting inflammation, killing bacteria, and controlling the smooth muscle tone of blood vessels and organs, but in large amounts, they cause oxidative stress (Reni, E 2018).

Because of an imbalance between free radical production and the antioxidant defense system in the body, free radicals will cause oxidative stress if there is an excess of them in the body, resulting in oxidative damage at the cellular level (Reni Yuslianti, 2017). Free radicals are byproducts of normal metabolic processes, and they are also known as Reactive Oxygen Species (ROS) (Winarsih, 2007). Free radical-induced oxidative stress has been linked to the emergence of several diseases, including diabetes, cardiovascular disease, rheumatoid arthritis, and various cancers (Phaniendra, A. et al, 2015). Antioxidants are required to prevent oxidative stress by inhibiting molecule oxidation (Ardhi, A. 2011). To neutralize ROS, our body cells produce both enzymatic and non-enzymatic antioxidants (Younus, H, 2018).

Indonesia has a diverse range of plants that can be used as raw materials for medicines, such as traditional medicines, herbal drinks, and herbs. *The World Health Organization* (WHO) recommended the use of traditional and herbal medicines for health, prevention, and treatment, particularly for chronic, degenerative diseases and cancer, in 2003. (Sari, L. 2006). It has recently piqued the interest of experts and researchers, particularly in the health sector, as evidenced by the search for potential antioxidant substances derived from nature, particularly plants. Herbs are plants whose plant parts are leaves, flowers, fruit, seeds, stems, wood, bark, wood, roots, rhizomes, or other plant parts that may be completely fragmented, according to the WHO definition. *Aia tempayang* drink is one of many medicinal plants (herbs), fruits, and vegetables that contain antioxidant active substances that function to boost the immune system and fight free radicals. *Aia tempayang* is a traditional West Sumatera drink made with spices like sappan wood, pangdahai, and basil seeds. When drank, *aia tempayang* has a bland flavor. In West Sumatera, *aia tempayang* is simple to find and can be purchased in packs for a reasonable Rp. 1,000. The local community typically brews *aia tempayang* directly with warm water. *Aia tempayang* is consumed by the local community because it is thought to reduce fever and treat cancer sores. Bred sappan wood, pangdahai, and basil seeds are left in the water for a few minutes until it turns reddish.

Sappan wood (*Caesalpinia sappan L*) is one of the antioxidant-rich components of the aia tempayang drink. According to Lim et al. (1997), the red dye in sappan wood is a brazilin group compound, which is an antioxidant compound with catechol in its chemical structure and can protect the body from free radical poisoning. In his research, Utari (2017) stated that sappan wood extract has an IC_{50} of 15,690 ppm. According to Palupi et al. (2015), the sappan wood extract to water ratio used is 1:10. According to Abidusy's (2017) research on the effect of sappan wood (*Caesalpinia sappan L.*) and cinnamon (*Cinnamomum burmanii BI*) proportions on the antioxidant activity of "Wedang Semanis" using a method consisting of two factors, namely factor 1 the proportion of cinnamon (K) consists of two levels, namely 3 g and 6 g. Factor 2: The proportion of sappan wood (S) is divided into three levels: 2g, 5g, and 8g. The DPPH test (*2,2-diphenyl-1-picrylhydrazil*) results showed that the K1S3 treatment had very strong antioxidant activity (3 g cinnamon and 8 g sappan wood) with a very strong IC_{50} parameter of 39.80 g/ml. Natural antioxidants can protect the body from reactive oxygen species damage and can inhibit lipid peroxidation in food. In recent years, there has been an increase in interest obtaining natural antioxidants (Syakur A. 2017).

Pangdahai known as *scaphium macropodum*, is similar to *schaphium affine*, which is known to have medicinal properties, one of which is recognized by ethnic leaders. This fruit contains alkaloids, glycosides, flavonoids, tannins, and saponins that can help with a variety of ailments such as heartburn, constipation, hemorrhoids, fever, and joint pain. This plant is known as the *Merpayang* plant by the Penghulu-ethnic group. However, as a result of modernization and the widespread use of generic drugs, the use of plants as medicine has declined. This fruit contains amino acids and flavonoids that can be used as antioxidants against mutagens that affect the stomach directly. Antioxidant activity of pangdahai 2,2-diphenyl-1-

picrylhydrazyl extract as a radical scavenger and reducer of potassium ferricyanide [K3Fe(CN)6]. Ogale and Lam et al found that the methanol extract of Pangdahai had high free radical scavenging activity, with IC_{50} values of 11.02 µmol/L and 98-200 µg/mL.

Basil seeds, also known as *Ocimum basilicum*, have traditionally been used for heartburn, dyspepsia, diarrhea, therapeutic purposes, increasing blood circulation, reducing inflammation, reducing cholesterol oxidation, improving immune function, and controlling blood sugar levels. Basil seeds contain a variety of compounds, including flavonoids, alkaloids, phenols, and essential oils. According to Warsi 2017, chemical compounds of ethanol extract and ethyl acetate fraction from basil seeds have antioxidant potential. The antioxidant ethyl acetate fraction (389.00±1.00) had a higher IC_{50} value than the ethanol extract (1,374.00±6.20) but was less potent than quercetin (2.10±0.01µg/mL) (Warsi and Sholichah, A. 2017). There have been few studies and publications on the synergistic properties of various herbal plants, and there has been no scientific study on *aia tempayang* drinks, so researchers are interested in testing the antioxidant activity of *aia tempayang* drinks using the DPPH method (*2,2-diphenyl-1- picrylhydrazyl-s-triazine*). This study will determine the antioxidant activity of *aia tempayang* drink as scientific evidence for people to feel the benefits of *aia tempayang* drink.

METHOD

Chemical and Materials

Sappan wood, pangdahai, and basil seeds were obtained in dry form from traditional markets in Solok City, as well as 2,2-diphenyl-1-picrylhydrazyl-s-triazine or DPPH (Sigma Aldrich), ethanol, and aqua dest. A UV-Visible spectrophotometer (Shimadzu), a microplate reader (FLUOstar Omega), a micropipette (Socorex), a micropipette tip, a digital scale, a thermometer, a vortex, a test tube (Iwaki), a beaker, a 10 ml volumetric flask, a 250 ml volumetric flask, a dark bottle, aluminum foil,

Preparetion *Aia Tempayang* Drink

The preparation of the *aia tempayang* drink is carried out by combining the three ingredients, weighing according to each dose variation, then brewing with warm water at 70^0C for 20 minutes because the main substances such as brazilin contained in sappan wood best survive at that temperature. *Aia tempayang* are stirred and left until they turn a reddish color. After that filtered. Likewise with the test preparation of each ingredient brewed with the same treatment. Some samples were made based on the dose I with a concentration of 0.65 g/l (S: 4 g, KS: 0.67 g, BS: 2.5 g) , dose II with a concentration of 1.29 g/l (S: 8 g, KS: 1.34 g, BS: 5 g), dose III with a concentration of 2.58 g/l (S: 16 g, KS: 2.68 g, BS: 10 g), dose IV with a concentration of 5 ,15 g/l (S : 32 g , KS : 5.36 g, BS : 20 g), and dose V with a concentration of 10.32 g/l (S : 64 g , KS : 10.72 g, BS : 40 g). The sample solution of *aia tempayang* based on each ingredient was made into sappan wood, made into 3 doses, namely the first dose of 72 mg, the second dose of 144 mg, and the third dose of 288 mg with a concentration of 0.36 g/l, 0.72 g/l. and 1.44 g/l, pangdahai was made into 5 doses, namely the dose I of 12 mg, dose II of 24 mg, the dose III of 48 mg, the dose IV of 96 mg, and the dose V of 192 mg with a concentration of 0.06 ; 0.12 ; 0.24, 0.48 ; 0.96 g/l, basil seeds were made into 5 doses, namely the dose I of 45 mg, dose II of 90 mg, dose III of 180 mg, dose IV of 360 mg, and dose V of 720 mg where the concentration was 0.23; 0.45 ; 0.90 ; 1.8 g/l, and 3.60 g/l. The following picture below shows the appearance of the components of *Aia Tempayang* and it was drinks:

(a) (b)

Figure 1: (a) ingredients of *aia tempayang*, (b) *aia tempayang* drink

Preparation of DPPH solution

Weigh the DPPH powder as much as 1 mg, then put it in a 50 ml measuring flask and add enough ethanol to the limit.

The DPPH Method is used to Test Antioxidant Activity

According to Hasanah M (2017), the test procedure was as follows: prepare a DPPH solution by weighing 1 mg of DPPH powder, then place it in a 50 ml measuring vessel and fill it with ethanol to the limit. *Aia tempayang* brewed with warm water have concentrations of 0.65 g/l, 1.29 g/l, 2.58 g/l, 5.15 g/l, and 10.32 g/l according to doses I, II, III, IV, and V. Concentration and water of *aia tempayang* dissolved in ethanol according to doses I, II, III with concentrations of 0.65, 1.29, and 2.58 g/l. Pipette 0.4 ml of the sample solution of *aia tempayang* that has been brewed with warm water, as well as material *aia tempayang* that has been dissolved in ethanol and the sample solution for each ingredient, into a 10 ml volumetric flask and fill with ethanol. Make the solution as homogenous as possible. Pour the solution into a test tube labeled with the treatment code, and vortex for 10 seconds. Pipette 0.2 ethanol, then add 3.8 ml of DPPH solution and vortex for 1 minute to make the blank solution. Pipette one sample into a 0.2 ml test tube, then add 3.8 ml of 0.05 Mm DPPH solution and vortex for 1 minute. The blank solution, crock-water *aia tempayang* samples, and solution of each ingredient were incubated in a dark place at 37°C for 30 minutes. The absorption was then measured using a microplate reader with a 517 nm wavelength. The *Aia Tempayang*, which had been dissolved in ethanol as a control, was treated in the same way as the sample. The following formula can be used to calculate the percentage of DPPH free radical binding:

$$\%\ Antioxidant\ Activity = \frac{blank\ absorbance - \ sample\ absorbance}{blank\ absorbance} \times 100\%$$

Data Analysis

The percentage of damping capacity was calculated using the DPPH antioxidant activity test method's calculation results. The concentration curve was plotted against the percent immersion capacity, and the regression equation y = a + x was obtained. The IC_{50} value is used to determine what concentration of the sample is needed to achieve 50% immersion capacity.

RESULT AND DISCUSSION

Aia tempayang, a combination of sappan wood, pangdahai, and basil seeds, has been shown to have health benefits and the potential to contain antioxidants. Antioxidants are compounds that slow down or prevent the oxidation process, while antioxidants are compounds that can prevent oxidation reactions by stopping the chain reaction caused by the emergence of free radicals, according to Hudson B.J.F (1990). DPPH free radical scavenging is one of the antioxidant activity tests. The DPPH method was used in this study to assess antioxidant activity. DPPH can be used to stabilize samples in the form of solids or liquids when mixed with a substrate. This method is used to evaluate antioxidant activity and quantify the number of complexes of antioxidant radicals formed in compounds that act as free radical scavengers or hydrogen donors. The DPPH method is one of the most widely used tests because it is inexpensive, straightforward, and does not necessitate the use of sophisticated equipment. The IC_{50} parameter is used to determine antioxidant activity using the DPPH method, which is the sample concentration required to capture 50% of DPPH radicals (Sugiyanto, R 2011). The working principle of this method is the presence of hydrogen atoms from antioxidant compounds that bind to free electrons in radical compounds, causing a change from free radicals to non-radical compounds, which is marked by a color change from purple to yellow when reacting with antioxidants (Molyneux P, 2004; Vandar, G. et al. 2003).

Figure 1 shows the curve of the results of testing the antioxidant activity of *aia tempayang* drink brewed with warm water at a temperature of 70^0C and material *aia tempayang* dissolved in ethanol as a control comparison which was tested using the DPPH method. IC_{50} analysis was calculated based on the linear regression equation obtained by plotting the concentration of the test solution with the percent inhibition of DPPH as a parameter of antioxidant activity, where the concentration of the test solution was the abscissa, and the percent inhibition was the ordinate.

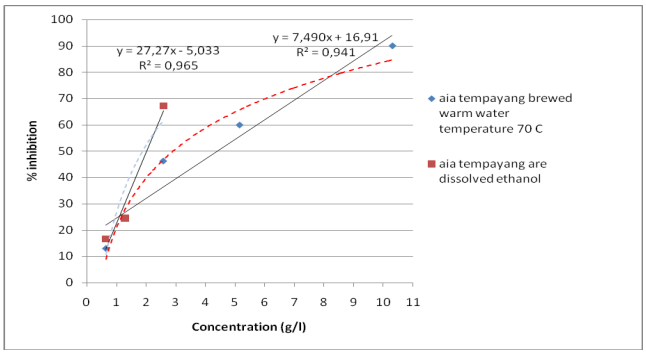

Figure 2.
The curve of the results of testing the antioxidant activity of *aia tempayang* drink and comparison

According to the graph above, the linear regression equation for *aia tempayang* brewed with warm water is y = 7.490 x + 16.91, while for the control comparison dissolved in ethanol, the equation is y = 27.27 x – 5.033. Table 1 below shows the results of testing the antioxidant activity/IC_{50} value of the *aia tempayang* drink with comparison:

Table 1
***Aia Tempayang's* antioxidant activity (IC50) test results**

Treatment	Dose	Equality	IC_{50} DPPH (g/l)
aia tempayang are brewed with warm water[a]	I : (KS 0.072 ; BJS 0.012 ; BS 0.045 g/200 gBB) = concentration 0.65 g/l II: (KS 0.144 ; BJS 0.024 ; BS 0.09) = concentration 1.29 g/l III : (KS 0.288 ; BJS 0.048 ; BS 0.18) = concentration 2.58 g/l IV : (KS 0.576 ; BJS 0.096 ; BS 0.36) = concentration 5.15 g/l V : (KS 1.152 ; BJS 0.192 ; BS 0.72) = concentration 10.32 g/l	y = 7.490 x + 16.91	4.417
aia tempayang are dissolved in ethanol[b]	I : (KS 0.072 ; BJS 0.012 ; BS 0.045 g/200 gBB) = concentration 0.65 g/l II : (KS 0.144 ; BJS 0.024 ; BS 0.09) = concentration 1.29 g/l III : (KS 0.288 ; BJS 0.048 ; BS 0.18) = concentration 2.58 g/l	y = 27.27 x – 5.033	2.018

[a]based on dry weight
[b]as a test control of antioxidant activity, inhibition

Table 1 shows the results of antioxidant testing of *aia tempayang* using the DPPH method, with an IC_{50} value of 4.417 g/l brewed with warm water, and an IC_{50} value of 2.018 g/l dissolved in ethanol, where the lower the IC_{50} value, the stronger the antioxidant activity. When the antioxidant activity of ethanol-diluted *aia tempayang* was compared to hot water brewed *aia tempayang*, it was discovered that the ethanol-diluted *aia tempayang* material had higher antioxidant activity. Because the materials used to dissolve in a specific solution, the results of the DPPH test are dependent on the test conditions used, such as the concentration of the extract, the initial concentration of the DPPH solution, the incubation time, and the solvent used. According to the findings of this study, *aia tempayang* brewed with warm water reduced free radicals by up to 50% at a concentration of 4.417 g/l, with this result evenly distributed between doses 3 and 4, while material *aia tempayang* dissolved in ethanol solvent reduced radicals by

up to 50% at a concentration of 2.018 g/l. This concentration falls somewhere between doses 2 and 3.

Sappan water extract has a higher antioxidant index than commercial antioxidants butylhydroxytoluene (BHT) and tertiary butyl hydroxy quinone (TBHQ), according to research by Lim et al. (1997), indicating that it has potential as a radical scavenging agent. without cost (Lim et al. 1997). Because their performance is similar to that of natural vitamin E, both BHT and TBHQ are synthetic compounds with high antioxidant activity (Rahmatiyah, R. 2012). According to Krisnawati's research, the antioxidant activity of the two groups differed significantly. The best treatment was 95 percent ethanol solvent, which had a total value of the antioxidant activity of 43,617 ppm, while distilled water extraction had a total value of 51,634 ppm. Furthermore, according to a study conducted by Sari R in 2016, ethanol extract of sappan wood has a superoxide-free radical scavenging power of up to 100%. Musthikaningtyas (2015) found that the addition of ginger filtrate and sappan wood filtrate to the manufacture of functional drink (*Eugenia polyantha bay leaf*) yielded the highest total phenol value when compared to the total phenol value of bay leaf filtrate and ginger filtrate. Tannins in Sappan wood are very high and are the dominant component in Sappan wood polyphenols, so the higher the addition, the higher the total phenol value (Palupi, Musthiksningtyas 2015).

Flavonoid compounds have antioxidant activity that can increase self-defense from diseases induced by free radicals. Flavonoid compounds are also known to reduce the risk of chronic diseases such as heart disease, diabetes, and cancer. The choice of solvent used for extraction must be appropriate to attract the desired compound (Firdiyani, F. et al, 2015). The solvent will more easily attract extracts with the same polarity (Sarastani, D 2002). Ethanol has a high polarity so that it can extract polar compounds, including phenolic compounds, steroids, terpenoids, alkaloids, and glycosides (Dia, Nurjanah, Jacoeb 2015). Ethanol also has a high boiling point and is non-toxic so it is safe to use (Aziz, T., Febrizky, S., Marion. 2014). The presence of ROS can be reduced and controlled by compounds that have antioxidant activity and anti-oxidative enzymes. Antioxidant activity can keep intracellular cells maintaining ROS levels at low levels (Shebis, Y. et al, 2013). The IC_{50} test in the form of *aia tempayang* has never been tested for the results of antioxidant activity in the form of steeping or dissolved in a solvent, but preliminary research has tested the antioxidant activity of each ingredient of *aia tempayang* consisting of sappan wood, pangdahai, and basil seeds. The following are the results of the antioxidant activity of each of the tested materials.

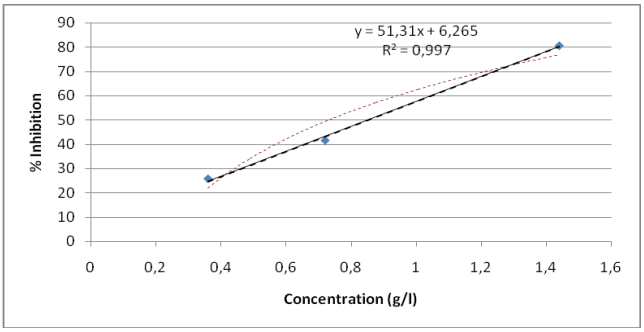

Figure 3.
Curve of the results of testing the antioxidant activity of sappan wood which is brewed with warm water at 70^{0}C for 20 minutes

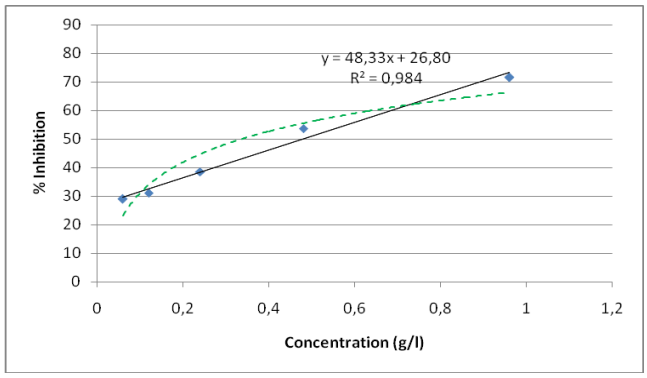

Figure 4.
Curve of the results of testing the antioxidant activity of pangdahai brewed with warm water at 70^{0}C for 20 minutes

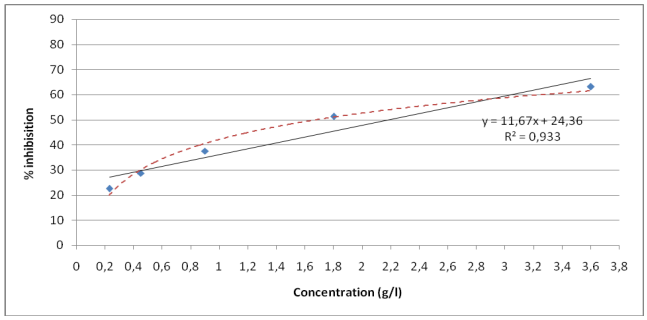

Figure 5
Curve of the results of testing the antioxidant activity of basil seeds brewed with warm water at 70^{0}C for 20 minutes

The results of the linear regression equation for each ingredient that is brewed with warm water are a cup, sappan wood y = 51.31 x + 6.265, pangdahai y = 48.33 x + 26.80, and basil seeds, y = 11.67 x + 24.36, as shown in the diagram above.

Table 2
The results of the antioxidant activity of brewing *Aia Tempayang* for each ingredient

Ingredients	Concentration (g/l)	IC_{50} (g/l)
Sappan wood Dose 1 (72 mg)	0.36	
Sappan wood Dose 2 (144 mg)	0.72	0.852
Sappan wood Dose 3 (288 mg)	1.44	
Pangdahai Dose 1 (12 mg)	0.6	
Pangdahai Dose 2 (24 mg)	0.12	
Pangdahai Dose 3 (48 mg)	0.24	0.480
Pangdahai Dose 4 (96 mg)	0.48	
Pangdahai Dose 5 (192 mg)	0.96	
Basil seeds Dose 1 (45 mg)	0.23	
Basil seeds Dose 2 (90 mg)	0.45	
Basil seeds Dose 3 (180 mg)	0.90	2.197
Basil seeds Dose 4 (360 mg)	1.8	
Basil seeds Dose 5 (720 mg)	3.6	

According to table 2 pangdahai had the highest IC_{50} antioxidant activity of 0.480 g/l, indicating that the lower the IC50 value, the greater the antioxidant activity. Sappan wood had an IC_{50} of 0.852 g/l after being brewed with warm water and left for 20 minutes at 70^{0}C, indicating that it could

reduce free radicals by up to 50% with 0.852 g/l. At dose 3, an IC_{50} of 0.480 g/l was obtained from a bowl flower that had also been soaked for 20 minutes. At doses 3 and 4, basil seeds soaked in warm water at 70°C for 20 minutes had an IC_{50} of 2.197 g/l.

The results of Abidusy's research (2017) related to the proportions of sappan wood and cinnamon in "Wedang Semanis" showed that the treatment of 3 g of cinnamon and 8 g of sappan wood with the IC50 parameter of 39.80 g/ml was very strong, meaning that as much as 8 g already showed as much as 50% of sappan wood. can scavenge free radicals. A total of 8 g of sappan wood consumed by humans is equivalent to 0.144 g or 144 mg when converted to rats. The results of the research by Dhage et al. (2015) and Surapantahanakorn also reported that intraperitoneal administration of 30-300 mg/kg ethanol extract of pangdahai showed anti-inflammatory properties such as significant dose-dependent inhibition of carrageenan-induced rat paw edema. Dhage et al. also discovered antioxidant activity using DPPH-reducing potassium ferricyanide [K3Fe (CN)6]. The results showed that pangdahai ethanol extract at 10-200 g/ml could capture DPPH radicals at EC concentrations of 800 ppm (Dhage P, Kasture SB, M.M 2015). In comparison to ethyl acetate and acetone extracts, the percentage of antioxidant activity measurements of the DPPH radical inhibition activity method of semi-polar and polar basil seed extracts (ethyl acetate, acetone, methanol) showed that methanol extract had the highest inhibitory ability against DPPH free radicals in all concentrations. At a concentration of 100 g/ml, the ethyl acetate solvent had the greatest inhibitory ability, followed by acetone at 100 g/ml and methanol at 80 g/ml (Nurcahyanti, A.D 2011).

CONCLUSION

Aia Tempayang, a combination of sappan wood, pangdahai, and basil seeds, has antioxidant activity that is lower when dissolved in warm water at 70^0 degrees Celsius for 20 minutes than when dissolved in ethanol. The low antioxidant activity of water dissolved in warm water is suspected because it is dependent on the solvent used, given that the antioxidant compounds in each ingredient dissolve in different types of solutions.

Funding

This work was supported by The Faculty of Medicine, Diponegoro University through the Research Development and Research (RPP) grant program

Conflict of interest

This research does not include any conflict of interest

REFERENCES

Ardhi, A. M. Radikal Bebas dan Peran Antioksidan dalam Mencegah Penuaan. *Sci. J. Pharm. Dev. Med. Appl.* 24, 4–9 (2011).

Aziz, T., Febrizky, S., Mario, A. D. Pengaruh Jenis Pelarut Terhadap Persen YIELD ALKALOID dari Daun Salam India (Murayya koenigii). *Kim. Tek.* 20, 1–6 (2014).

Dhage P, Kasture SB, M. M. Analgesic, anti-inflammatory, antioxidant and antiulcer activity of ethanolic extract of Sterculia Scaphigera Hance (Sterculiaceae) seeds in mice and rats. *Int J Biol Pharm Res* 4, 35–45 (2015).

Dia, S.P.S., Nurjanah, Jacoeb & A.M. Komposisi Kimia dan Aktivitas Antioksidan Akar, Kulit Batang dan Daun Lindur. *JPHPI* 18, 209 (2015).

Firdiyani, F., Agustini, W, T., Ma'ruf & W.F. Ekstraksi Senyawa Bioaktif sebagai Antioksidan Aami Spirulina platensis Segar dengan Pelarut yang Berbeda. *JPHPI* 18, (2015).

Kawk, H. W., Nam, G. H., Kim, M. J., Kim, S. Y. & Kim, Y. M. Scaphium affine Ethanol Extract Induces Anoikis by Regulating the EGFR/Akt Pathway in HCT116 Colorectal Cancer Cells. *Front. Oncol.* 11, 1–12 (2021).

Lim *et al.* Antioxidative activity of some solvent extract from Caesalpinia sappan Linn. *Korean J. Food Sci. Tech.* 28, 77–82 (1997).

Molyneux, P. The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakarin J. Sci. Technol.* 26, 211–219 (2004).

Nurcahyanti, A. D. R., Dewi, L. & Timotius, K. H. Antioxidant and Antibacterial Activities from Polar and Non Polar Basil (Ocimum sanctum Linn) Seed Extracts. *J. Teknol. dan Ind. Pangan* 22, 1–6 (2011).

Palupi, Musthikaningtyas Retno, T. D. W. Making Functional Drink Liang Tea Bay Leaves (Eugenia polyantha) with Addition of Ginger Filtrate and Secang Wood Filtrate. *Hurnal Pangan dan Agroindustri* 3, 1458–1464 (2015).

Phaniendra, A., Jestadi, D. B. & Periyasamy, L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian J. Clin. Biochem.* 30, 11–26 (2015).

Rahmatiyah, R. Penggunaan butil hidroksi toluen untuk menghambat ketengikan minyak kelapa hasil olahan petani. *J. Mat. Sains dan Teknol.* 13, 87–93 (2012).

Reni Yuslianti, Euis. 2018. *Pengantar Radikal Bebas dan Antioksidan*. Sleman. DeePublish.

Sarastani, D., S.T., S., T.R., M., Fardiaz, D. & Apriyantono, A. Aktivitas antioksidan ekstrak dan fraksi ekstrak Biji Atung (Parinarium glaberrimum Hassk.). *J. Teknol. dan Ind. Pangan* 13, 149–156 (2002).

Sari, L. Pemanfaatan Obat Tradisional Dengan Pertimbangan Manfaat Dan Keamanannya. *Maj. Ilmu Kefarmasian* III, 1–7 (2006)

Shebis, Y., ILuz, D., Y.K., T., Dubinsky, Z. & Yehoshua, Y. Natural Antioxidant : Function and Sources. *Food Nutr. Sci.* 4, 634–649 (2013).

Sugiyanto, R. . Paparan zat potensial karsiogenik melalui MNPCE ASSAY Kayu Secang (Caesalpinia Sappan L) dalam Upaya Prevensi Kerusakan DNA. (Universitas Gadjah Mada, 2011).

Utari, F. D., Sumirat, S. & Djaeni, M. Produksi Antioksidan dari Ekstrak Kayu Secang (Caesalpinia sappan L.) Menggunakan Pengering Berkelembaban Rendah. *J. Apl. Teknol. Pangan* 6, 1–4 (2017).

Vardar-Ünlü, G. *et al.* Antimicrobial and antioxidant activity of the essential oil and methanol extracts of Thymus pectinatus Fisch. et Mey. Var. pectinatus (Lamiaceae). *J. Agric. Food Chem.* 51, 63–67 (2003)

Warsi & Sholichah, A. R. Phytochemical screening and antioxidant activity of ethanolic extract and ethyl acetate fraction from basil leaf (Ocimum basilicum L.) by DPPH

radical scavenging method. *IOP Conf. Ser. Mater. Sci. Eng.* 259, (2017)

Widayati, E. Oxidasi Biologi,Radikal Bebas, dan Antioxidant. *Maj. Ilm. Sultan Agung* 50, 26–32 (2012).

Winarsih. *Antioksidan Alami dan Radikal Bebas*. (Kanisius Jogjakarta, 2007).

Younus, H. Therapeutic Potentials of Superoxide Dismutase. *Int. J. Health Sci. (Qassim).* 12, 88–93 (2018).

 ISSN 2502-4825 (print), ISSN 2502-9495 (online)