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BOILING TIME-DEPENDENT STUDIES ON THE TOTAL FLAVONOIDS CONTENT AND ANTIOXIDANT ACTIVITIES IN DENDROPHTOE PETANDRA (L.) MIQ.

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ABSTRACT

Boiling time effect on total flavonoid content and antioxidant activity of *Dendrophthoe petandra* leaves were studied. *D. petandra* is a parasite plant that is widely used on Indonesian people for traditional medicine with boiling techniques. The dried leaves were boiled in water for 5, 10 and 15 minutes, and quantitative analysis was performed from each boiled water. Total flavonoid levels were analyzed using UV/Visible Spectrophotometry as quercetin equivalent (QE) and antioxidant activity using FRAP (Ferric Reducing Antioxidant Power) as ascorbic acid equivalent (AAE). The total flavonoid levels and antioxidant activity showed a considerable change. The boiled water of *D. petandra* leaves for 10 minutes showed the highest level of total flavonoid content (9.0098 ± 0.0340 ppm QE) and antioxidant activity (30.6792 ± 0,0270 ppm AAE), whereas the content of total flavonoids were 7.2350 ± 0.0299 ppm QE and antioxidant activity were 23.9533 ± 0,0486 ppm AAE showed the lowest results boiling for 15-minute. Total flavonoid and antioxidant activity of boiling time for 5 minutes increases to 10 minutes and then decreased at 15-minute. Therefore, it is improper to boil the *D. petandra* leaves more than 10 minutes.

Keywords: antioxidant activity; boiling time; *dendrophthoe petandra* leaves; total flavonoid conten

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INTRODUCTION

Clove parasite (Dendrophthoe petandra (L.) Miq) is a parasitic plant that lives on clove trees (Syzygium aromaticum) and is used by Indonesian people as an alternative treatment. The marker compounds in the clove leaf parasite that are responsible for its activity are flavonoids which are a source of natural antioxidants (Ikawati et al., 2008). Antioxidant potential in aqueous extract and ethanol extract showed strong antioxidant potential with IC50 values of 11.4 g/mL and 6.8 g/mL, respectively (Fitrilia, 2015).

Boiling is a extraction method that is often used by the community in using natural ingredients as traditional medicine, but it can cause the loss of compounds that are not sensitive to heat and reduce nutritional quality. Boiling is cooking ingredients in boiling water. In the boiling technique, the boiling time needs to be controlled so as not to reduce the flavonoid content of the clove leaf parasite so that its antioxidant potential does not decrease. Clove parasites contain the aglycone flavonoid quercetin compound of 13.7021% QE but the content decreases to 0.2819% QE after boiling (Lekal & Watuguly, 2017). The decrease in the content of flavonoid compounds due to boiling resulted in decreased antioxidant potential. The presence of heating in boiling is responsible for the occurrence of oxidation,

thermal degradation, and loss of bioactive compounds from fresh vegetables. Different heating conditions have different effects on the antioxidant properties of vegetables (Kamalaja et al, 2018). To obtain maximum health utilization, clove parasites should be boiled using appropriate boiling conditions.

METHOD

The type of research used in this research is quantitative research. The population in this study were 180 hemodialysis patients with a total sample of 125 patients undergoing hemodialysis. The sampling technique used was purposive sampling which was determined based on the inclusion criteria and exclusion criteria. The data analysis used was univariate analysis to determine the characteristics of the patient's spirituality and bivariate analysis to determine the relationship between the two variables. The data collection technique used a spirituality research instrument, namely the Spiritual Well-Being Scale (SWBS). The SWBS developed by Polutzian and Ellison includes 20 question items consisting of 2 subscales, namely religious well-being (RWB) and existential well-being (EWB). The Likert scale that is assessed has 6 points with the results of the validity and reliability test being Cronbach's alpha of 0.82. While the resilience research instrument is the Connor and Davidson Resilience Scale (CD-RISC). The CD-RISC developed by Conner and Davidson includes 25 question items consisting of 3 subscales, namely tenacity, strength and optimism. The Likert scale that is assessed has 5 points with the results of the validity and reliability test being Cronbach's alpha of 0.927.

RESULTS

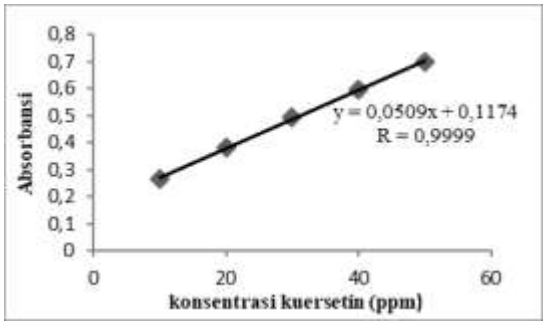
Clove parasite leaves Dendropthoe pentandra (L.) Miq. taken in Mereng Village, Tlobo Village, Jatiyoso District, Karanganyar Regency. The leaves taken were dark green leaves because the old leaves had a significant effect on the total levels of flavonoids, which are bioactive compounds that act as antioxidants. In the preparation of the material, the leaves are sorted first, then washed with running water to remove the dirt that sticks to the leaves. Drying is carried out until the parasite leaf tea is obtained as shown in Figure 1.

A B

Figure 1. Clove parasite leaves (Dendrophthoe petandra) Ket. (A) Fresh clove parasite leaves (B) Clove parasite leaf simplicia

Table 1.
Evaluation of boiled water with variations in cooking time

Quality	Waktu perebusan		
	5 minutes	10 minutes	15 minutes
Solution color	pale yellow	yellow	intense yellow
Yield	82%	70%	63%
Flavonoids			
Alkaline test	positive	positive	positive
Wilstatter Test "Cyanidin"	positive	positive	positive



Gambar 2. Kurva Regresi Linear Kuersetin dengan Rumus Regresi linier y = 0,0509x+ 0,1174 dan nilai r = 0,9999

Table 2.
Total flavonoid content of boiled water of parasite clove leaves with variations in cooking time

Boiling time	Triplo	Aborbansi	Kadar flavonoid total (ppm)	Rata-rata kadar flavonoid (ppm)
5 minutes	1	0,535	8,2043	8,2436 ± 0,0393
	2	0,537	8,2436	
	3	0,539	8,2829	
10 minutes	1	0,574	8,9901	9,0098 ± 0,0340
	2	0,575	8,9902	
	3	0,578	9,0491	
15 minutes	1	0,487	7,2612	7,2350 ± 0,0299
	2	0,486	7,2416	
	3	0,484	7,2023	

In this study, the standard reference solution used was ascorbic acid with a linear regression curve shown in Figure 4.

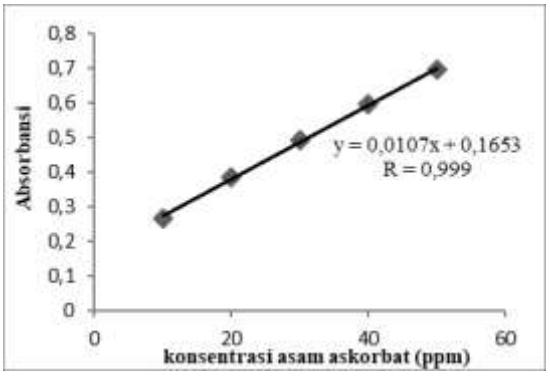


Figure 3. Ascorbic Acid Linear Regression Curve with Linear Regression Formula y = 0.0107+ 0.1653 and the value of r = 0.9999

Table 3.
Antioxidant activity of parasite clove leaf boiled water with variations in cooking time

Boiling time	Triplo	Aborbansi	Kadar flavonoid total (ppmAAE)	Rata-rata kadar flavonoid (ppmAAE)
5 minutes	1	0,4530	26,8879	26,8879 ± 0,000
	2	0,4530	26,8879	
	3	0,4530	26,8879	
10 minutes	1	0,4934	30,6636	30,6792 ± 0,0270
	2	0,4934	30,6636	
	3	0,4939	30,7103	
15 minutes	1	0,4210	23,8971	23,9533 ± 0,0486
	2	0,4219	23,9813	
	3	0,4219	23,9813	

DISCUSSION

Chopped fresh leaves with a size of 2-3 cm which aims to speed up the drying process. In addition, elongation can also affect the total flavonoid content produced because the smaller the leaf size, the wider the surface area so that the levels obtained will be more optimal. This is in line with research conducted by Tambun et al., (2016) that leaf size can affect the levels of flavonoids produced. In the preparation of dry leaves, the leaves are dried in an oven at 30oC, drying at a temperature of 300C so that the secondary metabolites contained in the leaves are not damaged. This drying also aims to remove the water content in the sample to avoid the proliferation of microbes, resistant to storage in the long term.

Clove parasite leaf decoction with variations in boiling time resulted in different qualities (Table 1). The longer the boiling time, the boiled water obtained showed the color of the solution was getting thicker and the yield of the boiled water decreased. This happens because the longer the heating process there will be an increase in the solubility of compounds in the material and evaporation of the cooking water will occur**.**

The results of the qualitative test of flavonoids showed that the boiled water of parasite clove leaves with variations in boiling time was positive for flavonoids (Table 1). In the alkaline test the sample was added with NaOH to produce a more intense yellow color. This is due to the decomposition by bases into yellow acetaphenone molecules due to the breaking of bonds in the isoprene structure (Hanani, 2015). In the Wilstatter test "Cyanidin" if a sample contains flavonoids, it is marked by a color change from yellow orange to red. In this test, the addition of HCl is intended to detect flavonoid compounds to hydrolyze flavonoids into their aglycos, namely by hydrolyzing O-glycosyl into aglycones (not bound to sugar) so that H+ is released and O is bound by Mg so that the reduced flavonoids with Mg and HCl powder give a color. red, yellow, or orange (Markham, 1988). The test results on the three boiled water samples resulted in a change in the color of the solution to intense red, so it was concluded that it contained flavonoids.

Determination of the levels of flavonoids contained in the parasite clove leaf was carried out by UV-Visible spectrophotometric method using aluminum chloride with the principle of forming a stable complex between aluminum chloride and the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom of the flavone group. and flavonols. In addition, the formation of a labile complex on the ortho-hydroxy in ring B. The quercetin standard used is a flavonoid of the flavonol group which has a keto group on the C-4 atom and also a hydroxyl group on neighboring C-3 and C-5 atoms and has an ortho-hydroxyl

group on the C-4 atom. ring B resulting in a complex reaction between quercetin and AlCl3 (Markham, 1988). The formation of the quercetin aluminum chloride complex is indicated by a yellow solution so that there is a shift in the wavelength towards the visible (visible light) and the addition of potassium acetate serves to stabilize the complex compound formed (Chang et al. 2002).

Determination of the wavelength used for quantitative analysis is the wavelength that has the maximum absorbance so that it shows maximum sensitivity. Based on the results of determining the maximum wavelength of quercetin obtained a wavelength of 439.5 nm. Furthermore, the measurement of Operating Time is carried out which is the time so that the flavonoid compounds react with AlCl3 optimally. Based on the results of measurements carried out every minute for 60 minutes, it shows the operating time of the quercetin complex in the 30th minute.

The standard curve was made with the aim of knowing the relationship between the concentration of the solution and its absorbance value so that the concentration of the sample can be known. If the Lambert-Beer law is fulfilled, the curve is a straight line (Grace et al, 2015). Based on the standard linear regression curve of quercetin in Figure 2, the linear regression equation y = 0.0509x + 0.1174 is obtained with a value of r = 0.9999. These results indicate the linearity of the relationship between concentration and absorbance of quercetin so that it can be used to measure total flavonoids in the test sample.

Total flavonoid content in boiling water with variations in boiling time is shown in table 2. Variations in boiling time resulted in differences in total flavonoid content in each treatment. The flavonoid content of herbal tea leaves of parasite cloves in 5-minute stew was 8.2436 ppm QE followed by an increase in levels in 10-minute stew of 9.0098 ppm QE which was the highest level, while in 15-minute stew decreased with total flavonoids of 7.2350 ppm QE. Based on parametric analysis using One Way Anova analysis, the significance value of flavonoid levels was 0.000 <0.05. This shows that there is a significant difference between total flavonoid levels in boiling water and variations in boiling time.

The antioxidant activity of the parasite clove leaf herbal tea was tested using the FRAP (Ferric Reducing Antioxidant Power) method. The FRAP method was chosen because it has a high sensitivity compared to DPPH and FIC (Maesaroh, et al. 2018). The DPPH and FRAP antioxidant activity test methods were found to be the most effective and efficient among the three test methods. While the FIC method is the least effective and inefficient because of its very low sensitivity. According to Benzie and Strain (1996), the FRAP method is a method for measuring antioxidant activity based on the ability to reduce ferric ions (Fe3+) to ferrous ions (Fe2+) at low pH. Where will form a blue complex which indicates the presence of antioxidant activity in the sample.

The antioxidant activity value of the boiled water of parasite cloves on the variation of boiling time showed the same effect as the total flavonoid content in each sample. Table III shows that the 5-minute stew obtained an average of (26.8879 ± 0.000ppm AAE) followed by an increase in activity in the 10-minute stew (30.6792 ± 0.0270ppm AAE), and decreased in the 15-minute stew (23.9533 ±0.0486ppm AAE). Based on parametric analysis using One Way Anova analysis, the significance value of antioxidant activity was 0.000 < 0.05. This shows that there is a significant difference in the effect of antioxidant activity in boiling water with variations in boiling time.

The boiling time of the clove leaf parasite has a significant effect on the total flavonoid content and antioxidant activity. This happens because the process of boiling the leaves can reduce the content of flavonoids in plants, one of which is flavonoids in the leaves of clove parasite. The reduced content of flavonoids is the underlying reason for the decrease in the value of the antioxidant activity of the clove leaf parasite. In a 15-minute stew, the flavonoid compounds will decrease so that the value of their antioxidant activity decreases. This shows that the higher the flavonoid content, the higher the value of antioxidant activity in the clove parasite leaves. The longer the heating time causes the levels of the compounds contained to decrease due to the nature of flavonoid compounds that are not resistant to heat (Saragih, 2014). This result is in line with the research of Loku et al 2001 which found that total flavonoids increased after heating at a certain temperature and amount of time, while heating for 3 hours at 150C decreased the flavonoid content. In addition, the total flavonoids in the leaves of Muntingia calabura showed a continuous decrease in boiling from 5 to 30 minutes.

The heating given in the extraction process can result in changes in the structural integrity and cellular matrix of the plant and this causes a positive effect in solubility and a negative effect on the phytochemical properties contained therein. The presence of damage to the cellular matrix can help penetrate the solvent in the pectin or cellulose network and make the compound more extractable into the solvent. According to Saikia and Mahanta, 2013 showed an increase in antioxidant activity with FRAP and phenolic content in vegetables. However, the application of excessive heat during cooking can cause significant changes in phenolics and flavonoids because they are very unstable compounds (Ismail, et al 2004). In the study, the boiling temperature was ±1000C which was marked by boiling water so that heating for too long and too high a temperature could cause degradation of compounds, thereby reducing the flavonoid content.

CONCLUSION

The boiling time of the clove leaf parasite affects the total flavonoid content and antioxidant activity. The best time to boil the parasite clove leaves was 10 minutes with total flavonoid content (9.0098 ± 0.0340 ppm QE) antioxidant activity (30.6792 ± 0.0270 ppm AAE) and decreased at 15 minutes with a total flavonoid of 7, 2350 ± 0.0299 ppm QE and antioxidant activity of 23.9533 ± 0.0486 ppm AAE showed the lowest yield at 15 minutes of boiling. Therefore, it is not recommended to boil the leaves of parasitic cloves for more than 10 minutes.

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