Advances in Social Science, Education and Humanities Research, volume 242
2nd International Conference on Vocational Education and Training (ICOVET 2018)

Study of Antioxidant Activity in Sinom Drinks From Breadfruit (Artocarpus Altilis) Leaves

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***Abstract*— Breadfruit leaves contain antioxidant including flavonoids and phenolics compound. This study aims to analyze the chemical characteristics (antioxidant capacity, phenolic and flavonoid content) of sinom drinks sourced from breadfruit leaves. The experimental design was employed by treatment of ratio between breadfruit leaves and tamarind leaves, which are 70% : 30%, 80% : 20%, 90% : 10%. Two-replication data was then analyzed by ANOVA and DMRT, after a DPPH assay in antioxidant activity, flavonoid and phenolic content. Results show that the highest antioxidant activity is reached by a ratio of 90%: 10% with IC50 of 66,600 ppm. The larger the concentration of breadfruit leaves extract is given, the greater the antioxidant activity. The trend was similarly occured in the phenolic and flavonoids contents; in which the highest values of 90%: 10% ratio are 1926,091 mg/L and 582,717 mg / L, respectively.**

Keywords*— antioxidant activity; sinom; breadfruit leaves; flavonoid; phenolic.

I. INTRODUCTION

Breadfruit leaves contain phytochemicals, such as saponins, polyphenols, hydrocyanic acid, potassium, acetylcolin, tannins, riboflavin, and phenol (Wang et al., 2007). Breadfruit leaves can be used in treating diabetes mellitus, malaria, diarrhea (Jagtap and Bapat, 2010). A study by Arung et al. (2009) shows that the extract of breadfruit wood can be used for cancer treatment. Artocarpus altilis is known for its nutritional, therapeutics, and medicinal properties. The medical use of Artocarpus altilis is abundant and unlimited. The leaves have been reported to reduce liver disease and fevers in Taiwan.

Breadfruit leaves contain flavonoids of 8-geranyl-4,5,7-trihydroxyflavone which serves as antidiabetic (Amarasinghe et al., 2008). Ethanol, ethyl acetate, and butanol contained in breadfruit leaves extract have the ability to inhibit an a-glucosidase enzyme (Badrie and Broomes, 2010).

In making sinom, the scent of breadfruit leaves is considered to be unpleasant. In order to eliminate the scent, cinnamon (Cinnamomum cassia) is added and serves as a natural aroma. The use of citric acid is substituted by Averrhoa blimbi because it contains a high level of natural Vitamin C of 25mg/100g and citric acid contained in Averrhoa blimbi is 92.6-133.8 meq acid/100 g total solid (Kurup and Mini, 2017). The phytochemicals contained in Averrhoa blimbi including oxalic, saponins, phenols, flavonoids (Baba et al., 2016). Saponins and flavonoids contained in Averrhoa blimbi can stimulate pancreas in producing and releasing insulin hormones, and regenerate damaged pancreatic beta cells in people with diabetes mellitus (Chang et al., 2013).

The parameter used to identify the antioxidant capacity is IC50 which is the maximum half-inhibitory concentration. The IC50 concentration of an antioxidant substance can cause 50% DPPH loses of radical character or concentration of an antioxidant substance that gives a 50% inhibition percentage. The material is classified as having very high antioxidant capacity if the value of IC50 is less than 50 ppm, high capacity if the value of IC50 is between 50-100 ppm, intermediate capacity if the value of IC50 is between 100-150 ppm, low capacity if the value of IC50 is between 150-200 ppm (Molyneux, 2004).

An antioxidant is a compound which is capable of binding free radicals and high reactive molecules, thus inhibiting the occurrence of cell damage (Pisoschi and

Negulescu, 2012). An antioxidant system as a mechanism of protection against free radical attack has naturally existed in our body. There are two types of antioxidants: internal and external antioxidants. An internal antioxidant is a self-produced antioxidant found in the SOD enzymes (superoxyde dismutase) that serves as a protector of damaged cells, also known as a primary antioxidant. The external antioxidant cannot be produced by the human body, but can be obtained from natural ingredients containing Vitamin A, Vitamin C, Vitamin E, and Flavonoids (Pisoschi and Negulescu, 2012). Antioxidants derived from food or obtained from the outside of human's body are called secondary antioxidant (White et al., 2014).

II. METHODE

The research is experimental research. The experimental design used in this study was a Complete Randomized Design (RAL). This research using three the ratio between breadfruit leaves with tamarind leaves of the 70%:30%, 80%:20%, 90%:10%. The materials used in the production process of breadfruit leaves functional beverage to include breadfruit leaves, tamarind leaves, turmeric, Averrhoa blimbi, palm sugar, and water. The materials used in the chemical analysis were methanol, DPPH solution, HNO3, and distilled water.

The observation was conducted on sinom from breadfruit leaves functional beverage, which included chemical analysis of the fresh ingredients and the breadfruit leaves a functional drink, namely to identify their antioxidant capacity (DPPH method). Solution DPPH 6 × 10í5 M obtained by dissolving the DPPH as many as 1,82 mg into 50 ml methanol. The solution obtained by dissolving substance test as many10 mg into 1 ml methanol. The solution included in a tube that is sheltered from light with pippet as many 33,33 ml and then added 1ml DPPH. A solution is shaken with vortex for 10 seconds. Next, incubation solution on the temperature 37 °C for 20 minutes. During the reduction process by an antioxidant, a solution of DPPH radical will change a shade of purple become pale yellow.

III. RESULT

The mean of antioxidant capacity identified by DPPH analysis method on sinom from breadfruit leaves can be seen in Table 1.

Table 1. The mean of antioxidant capacity in sinom from breadfruit leaves (IC50, ppm)

Samples (Breadfruit leaves)	Repetition		Mean (ppm)
	1	2	
70%	78,381	77,915	78,148
80%	70,312	69,981	70,147
90%	66,445	66,755	66,600

Based on Table 1, the highest IC50 value in sinom from breadfruit leaves is 90% (66,600 ppm), followed by 80% (70,147 ppm), and the lowest is 70%. Putri and Hidajati (2015) state that a compound is considered to have very high antioxidant capacity if the IC50 value is less than 50 ppm and high capacity is the IC50 value is between 50-100 ppm. The data were further analyzed using ANOVA, which can be seen in Table 2.

Table 2. ANOVA test result of Antioxidant capacity in sinom from breadfruit leaves

	Sum of Square	Df	Middle Square	F	Sig.
Among groups	139,969	2	69,985	991,563	0,000
In-group	,212	3	,071		
Total	140,181	5			

The ANOVA test result of antioxidant capacity in sinom from breadfruit leaves shows a significant difference in the antioxidant capacity with different percentages. The analysis was followed by DMRT test (Duncan's Multiple Range Test) to determine the level of antioxidant difference in sinom from breadfruit leaves. The result of the DMRT test can be seen in Table 3.

Table 3. DMRT test result of antioxidant capacity in sinom from breadfruit leaves

Duncan[a]

Samples (sinom)	N	Absolute value = 0.05		
		1	2	3
90%	2	66,60000		
80%	2		70,14700	
70%	2			78,14800
Sig.		1,000	1,000	1,000

The DMRT test shows that the antioxidant capacity at the 70% treatment is significantly different from the 80% treatment, the antioxidant capacity at the 70% treatment is significantly different from the 90% treatment, the antioxidant capacity at the 80% treatment is significantly different from the 90% treatment.

The Level of Phenol in Sinom from Breafruit Leaves

The mean of phenol identified by DPPH analysis method on sinom from breadfruit leaves can be seen in Table 4.

Table 4. The mean of phenol in sinom from breadfruit leaves

Sample (breadfruit leaves)	Repetition		Mean (mg/L)
	1	2	
70%	1567,000	1585,182	1576,091
80%	1707,909	1721,545	1714,727
90%	1917,000	1935,182	1926,091

Based on Table 4, the highest phenol is in the 90% sinom from breadfruit leaves (1576,091 mg/L), followed by the 80% sinom from breadfruit leaves (1714,727 mg/L), and the lowest is in the 70% sinom from breadfruit leaves (1926,091 mg/L). The data on total phenol were further analyzed using ANOVA, which can be seen in Table 5.

Table 5. ANOVA test result of total phenol in sinom from breadfruit leaves

	Sum of square	Df	Middel square	F	Sig.
Among groups	11628,310	2	5814,155	2108,875	,000
In-group	8,271	3	2,757		
Total	11636,581	5			

The result of the ANOVA test on total phenol of sinom from breadfruit leaves shows a significant difference in total phenol with different percentages. The following test is DMRT (Duncan's Multiple Range Test) to determine the level of total phenol difference in sinom from breadfruit leaves. The DMRT test result can be seen in Table 6.

Table 6. DMRT test result of total phenol in sinom from breadfruit leaves

Duncan[a]

Sample (sinom)	N	Absolute level = 0.05		
		1	2	3
70%	2	1576,09100		
80%	2		1714,72700	
90%	2			1926,09100
Sig.		1,000	1,000	1,000

The DMRT test shows that the total phenol at the 70% treatment is significantly different from the 80% treatment, the antioxidant capacity at the 70% treatment is significantly different from the 90% treatment, the antioxidant capacity at the 80% treatment is significantly different from the 90% treatment.

The Level of Flavonoids in Sinom from Breadfruit Leaves

The mean of flavonoids identified by DPPH analysis method on sinom from breadfruit leaves can be seen in Table 7.

Table 7. The mean of flavonoids in sinom from breadfruit leaves

Samples (breadfruit leaves)	Repetition		Mean (mg/L)
	1	2	
70%	474,022	477,283	475,653
80%	517,500	518,587	518,044
90%	581,630	583,804	582,717

Based on Table 7, the highest flavonoids are in the 90% sinom from breadfruit leaves (582,717 mg/L), followed by the 80% sinom from breadfruit leaves (518,044 mg/L), and the lowest is in the 70% sinom from breadfruit leaves (475,653 mg/L). The data on total flavonoids were further analyzed using ANOVA, which can be seen in Table 8.

Table 8. ANOVA test result of total flavonoids in sinom from breadfruit leaves

	Sum of square	Df	Middle square	F	Sig.
Among groups	11628,310	2	5814,155	2108,875	,000
In-group	8,271	3	2,757		
Total	11636,581	5			

The result of the ANOVA test on total flavonoids of sinom from breadfruit leaves shows a significant difference in total phenol with different percentages. The following test is DMRT (Duncan's Multiple Range Test) to determine the level of total flavonoids difference in sinom from breadfruit leaves. The DMRT test result can be seen in Table 9.

Table 9. DMRT test result of total flavonoids in sinom from breadfruit leaves

Duncan[a]

Samples (sinom)	N	Absolute level = 0.05		
		1	2	3
70%	2	475,65250		
80%	2		518,04350	
90%	2			582,71700
Sig.		1,000	1,000	1,000

The DMRT test shows that the total flavonoids at the 70% treatment are significantly different from the 80% treatment, the antioxidant capacity at the 70% treatment is significantly different from the 90% treatment, the antioxidant capacity at the 80% treatment is significantly different from the 90% treatment.

IV. DISCUSSION

A. *Antioxidant Capacity in Sinom from Breadfruit Leaves*

The analysis of the antioxidant capacity of sinom from

breadfruit leaves shows that the highest level is 90% with IC50 of 66,600 ppm. This indicates that the higher the percentage of the breadfruit leaves used, the higher the antioxidant capacity produced. The IC50 value indicates the amount of antioxidant capacity; the smaller the value of IC50 is the antioxidant capacity increases. The addition of breadfruit leaves percentage is directly proportional to the capacity of antioxidants produced; it means that if the percentage of breadfruit leaves increases, the antioxidant capacity will increase as well.

The composition in using breadfruit leaves affects the antioxidant capacity produced because breadfruit leaves contain antioxidants, such as flavonoids and phenols (Amarasinghe et al., 2008). The result of the phytochemical test shows that the ethanol extract of breadfruit leaves contains flavonoid, polyphenols, saponins, alkaloids, and tannins (Sairam, S. et al, 2013). In another study, it also identified that the methanol extract of dried breadfruit leaves contains flavonoids, phenols, saponins, and tannins (Maharani et al, 2014).

Suryanto and Wehantouw (2009) also state that the breadfruit leaves extract contains condensed phenol, flavonoids and tannins. In addition, the study shows a positive relationship between free radical capture with total antioxidant content from breadfruit leaves extract. The larger the concentration of breadfruit leaves extract is the greater the antioxidant activity.

B. The level of phenol in sinom from breadfruit leaves

The analysis of the highest phenol content is obtained by sinom from breadfruit leaves at 90% (1926,091 mg/L). It shows that the higher the percentage of breadfruit leaves used, the higher the phenol level. Phenol constitutes one of the compounds contained in breadfruit leaves. The phytochemical test of breadfruit leaves by Maharani et al. (2014) shows that dried breadfruit leaves extract contains phenol. Another study also shows the phenol content in breadfruit leaves extract with different total phenol content by using three different types of solvent media (Suryanto and Wehantouw, 2009).

Phenolic compounds are considered to be significant in terms of antioxidant activity; the greater the content of phenolic compounds is the greater the antioxidant activity (Shahwar et al, 2010). The phenol extract from breadfruit leaves contains high anti free radical activity and antioxidants. The phenol level in breadfruit leaves extract is directly related to the antioxidative activity; thus phenol also serves as antioxidants.

C. The level of flavonoids in sinom from breadfruit leaves

The analysis of the highest flavonoid content was obtained by sinom from breadfruit leaves at 90% (582,717 mg / L). It indicates that the higher the percentage of breadfruit leaves used the higher the level of flavonoids. Flavonoids constitute one of the compounds contained in breadfruit leaves (Sairam, S. et al, 2017).

A study by Utami et al. (2015) of phytochemical screening and breadfruit leaves extract shows the flavonoid compounds contained in it. Suryanto and Wehantouw (2009) assert that there is flavonoid content extracted from breadfruit leaves by using three different types of solvent. According to Jalal et al., (2015), there are two compounds of geranylated flavonoids from breadfruit leaves: 2-geranyl-2 ', 4', 3,4 tetrahydroxidehidrokolon, and 8-geranyl-4 ', 5,7 trihydroxyflavanone; both compounds are isolated from the methanol extract of breadfruit leaves. Flavonoids are potential compounds to serve as antioxidants (Jalal et al., 2015), so the high level of flavonoids in sinom from breadfruit leaves is proportional to the antioxidant capacity produced.

Various studies also state that flavonoids can serve as antidiabetic. (Badrie and Broomes, 2010) explains that the flavonoid compound and extract from breadfruit leaves is able to inhibit the activity of α-glucosidase enzyme. In testing the activity of decreasing glucose level in vitro (Fakhrudin , 2015), it shows the effect between the addition of ethyl acetate and methanol extract from breadfruit leaves and the ability of decreasing different glucose level. The greater the flavonoid level is the value of 50% decrease in glucose level is smaller meaning the better the activity of decreasing glucose level. A study by Jalal et al (2015) shows similar result of a decrease in blood glucose level in mice with breadfruit leaves water treatment; the decrease in blood glucose levels is due to the mechanism of flavonoid active substances contained in breadfruit leave.

V. CONCLUSION

Higher pencentage of breadfruit leaves used, stronger in antioxidant capacity of breadfruit leaf's functional drinks. This is because of phenol and flavonoid contents which is potential to use as antioxidant. The addition of breadfruit leaves also affects the phenol and flavonoid content. It can be assued that increasingly percentage of breadfruit leaves, the higher phenol and flavonoid contents in functional drinks.

VI. ACKNOWLEDGEMENTS

Authors thank to Universitas Negeri Malang for funding this research by PNBP Scheme.

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