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THE EFFECT OF BOILING TIME ON POLYPHENOL COMPOUNDS AND ANTIOXIDANT ACTIVITY OF CIPLUKAN (*PHYSALIS ANGULATA .L*)

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ABSTRACT

This study aimed to determine the effect of boiling time on polyphenol compounds and antioxidant activity ciplukan (leaves, stems, fruit, roots and mixture of each plant part). This research used Randomized Block Design (RBD) with five groups (leaves, stems, fruit, roots, and mixture of each part of the plant) and three treatments (10, 20 and 30 min). The analysis for the experiment were the antioxidant activity of each part of the plant by using various incubation time, the antioxidant activity and total polyphenol for each treatment. The results showed that the duration of boiling had a significant effect on total polyphenols but no significant effect on antioxidant activity. The highest total polyphenols were obtained on leaves with 30 min boiling duration (1210.5 mg GAE / g), and the highest antioxidant activity was also obtained on leaves with 30 min boiling time (88.32%).

Keywords: ciplukan (*Physalis angulata. L*), boiling, polyphenol, antioxidant, syrup

INTRODUCTION

Ciplukan (*Physalis angulata*, *L.*) is an annual crop of herbs from the Solanaceae family. This plant grows in the lowlands up to 1200 m above sea level, as a disturbing plant in the fields, gardens, shrubs and roadside. The Physical genus (Solanaceae) has about 90 species spread across tropical and subtropical countries around the world and has been widely recognized as traditional medicine. Chemical composition of ciplukan plants, including seeds contain 12-25% protein and 15-40% fatty oils with main components of palmitic acid and stearic acid, roots contained of alkaloid, leaves ontained of flavonoid, and buds contained of flavonoids and saponins (Fauzan *et al.,* 2018). Chemical content contained in ciplukan include saponins, flavonoids, polyphenols, chlorogenic acid, sugar substances, elaic acid, and fisalin. Polyphenols and flavonoids are included in phenol compounds, and are the main compounds in ciplukan plants that can be used as antioxidants. According to Anggraini et al. (2019), although the antioxidant is present in the food naturally, but if the ingredients cooked, the level will be reduced due to chemical and physical degradation.

Boiling is a cooking process in boiling water about 100 ° C, where water as a heat conducting medium. Boiling is done by soaking the ingredients in boiling water (100°C) with a various time depending on the properties, type, and size of the ingredients. Beside to cook the ingredients, boiling can also negatively affect

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some properties of food such as the occurrence of color degradation and the content of some vitamins are susceptible to high temperatures such as vitamin C and vitamin E. Cooking beside improving digestibility, taste and killing pathogen microorganisms, it also can affect nutrient content of the food.Ciplukan plants are generally used as a traditional medicine by the community. Processing as medicine is still relatively simple. Ciplukan process as medicine by boiling the whole ciplukan plants (including leaves, stems, fruit, and roots) in a closed pan. In boiling, the samples have to soak well in water and will finished when the extract is reduced. The infused water will have dark green color.

One of the factors which are affecting the extraction by boiling is extraction time. The principle is that the longer the extraction, the contact between the solvent and the material will be longer, so that from both materials' diffusion will occur until the concentration of the solution inside and outside in a balanced state (Zhang *et al.*, 2018). The process to make the ciplukan extraction still vary, so it will necessary to do research about boiling time in order to use ciplukan for medicine. The high temperature and length of boiling time can affect the content of a compound. Boiling time is expected to affect the bioactive components contained in ciplukan plants. Therefore, this research was conducted to determine the influence of boiling time on polyphenol compounds and antioxidant activity of boiled water of ciplukan plants (leaves, stems, fruits, roots and whole plants).

EXPERIMENTAL SECTION

Place and Time

The research was conducted in Central Instrumentation Laboratory of Agricultural Technology Faculty, Laboratory of Technology and Process Engineering of Agricultural Products, and Laboratory of Chemistry, Biochemistry of Agricultural Product and Food Nutrition, Agricultural Technology Faculty, Andalas University, Padang.

Materials and Tools

The main raw material in this research was ciplukan plant (Physalis angulata, L) taken directly around Padang City West Sumatera Province. The chemicals for the analysis used in this study were ethanol, aquades, DPPH, methanol, follin-ciocelteu reagents, Na_2CO_3 5%, citric acid, acetic acid, CMC, and dyes.

The tools used in this study were a set of beaker, cup, reaction tube, dropper, stirrer, funnel, vortex, aluminum foil, scales, water bath, glass bottle, refrigerator, stopwatch, spectrophoto- meter, thermometer.

Research Methods

The design used for this study was Randomized Block Design (RBD) with five groups and three treatments.

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The treatment provided for each group consisted of:

A: Boiling time of 10 min

B: Boiling time of 20 min

C: Boiling time of 30 min

The group was the difference parts of the plants, namely: leaves, stems, fruit, roots, and mixture of each part of the plant.

Implementation

Raw Material Preparation

Raw material used was ciplukan growth around Padang city, West Sumatra province. The collection of raw material done in the afternoon. Then the plants sorted and cleaned from dirt like soil and so forth.

Boiling of Ciplukan Plant

Selected parts of the ciplukan plant boiled, washed and drained for 3 min. Weighed 2.5 g of samples and then put into a glass cup containing 50 ml of water (0.5: 10 b / v). Boiling was done by using hot plate with temperature of 100°C. Boiling time was adjusted according to treatment (10, 20, and 30, min), and boiling water was ready for analysis.

Making of Syrup from Boiling Water of Ciplukan Plant

Boiling water that used as syrup heated with a 500 g of sugar and stirred constantly until it boiled with a temperature of 100°C. After boiled, the boiling water added 0.5 g of citric acid to regulate the acidity and added 0.3 g of CMC to be slightly thickened and added food dye of 0.5 g until thickened. This heating process done for 25 min to get a good syrup results. The syrup was filtered to get a better results. The syrup was put into a sterilized glass bottle that boiled for 15 min. After the packaging done, the syrup was pasteurized again so that the sterile syrup from microbes due to the previous packing process.

Observations

In this study observed the antioxidant activity of raw materials. For the boiling water, observation of total polyphenol and antioxidant activity, and organoleptic test on syrup product that were produced.

RESULTS AND DISCUSSION

Antioxidant of Raw Material

Raw material that used in this study was the whole parts of ciplukan plants (*Physalis angulata L.*) including leaves untill its roots. In antioxidant analysis of raw material done 4 different time for sample storage in a dark room that were 5, 15, 30, and 45 min. The aim was to know if the antioxidant activity in ciplukan plant include in "*slow-acting*" or "*fast-acting*" material. Analysis result of antioxidant activity of

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raw material in diferent time can be seen in Table 1, and the best time to obtain the highest antioxidant activity can be seen in Table 2.

Table 1. Analysis Result of Antioxidant Actifity of Raw Material

Material	Antioxidant Activity (%)			
	5 min	15 min	30 min	45 min
Leaves	33.71	30.26	42.22	40
Fruits	16.6	13.05	25.15	15.51
Stems	57.21	75.65	78.53	87.52
Roots	47.94	39.29	44.02	49.93
Mixture *	31.73	39.1	40.95	38.25

Table 2. The Best Time to Obtained The Highest Antioxidant Activity

Analysis	Antioxidant Activity (%)	Best Time (min)
Leaves	42,22 ± 0,14	30
Fruits	25,15 ± 0,04	30
Stems	87,52 ± 0,02	45
Roots	49,93 ± 0,14	45
Mixture *	40,95 ± 0,05	30

Note : (±) Standard of Deviasion

* Mixture = Mixture of Leaves, Fruits, Stems, dan Roots

The highest antioxidant activity of the ciplukan plant was found in the stem and the lowest was in the fruit. The best time ranged from 30-45 min indicated that the antioxidants contained in ciplukan plants need considerable time to bind to free radicals. So antioxidant in ciplukan plant classified as *slow-acting*. The reaction mechanism between antioxidants and DPPH depends on the structural form of antioxidants. Some compounds reacted very quickly with DPPH, but most of the compounds tested reacted more slowly and the mechanism looks more complex (Yusuff, *et al.,* 2019). Based on Anggraini (2011) the reaction time of 30 min resulted in an antioxidant activity higher than 2 min, the difference indicating the optimal reaction of catechins and slow acting antioxidants in gambier. The best time that was obtained used on antioxidant activity analysis of boiling water of ciplukan plant.

Observation of Boiling Temperature of ciplukan

Total Polyphenols

Total polyphenols are a group of chemicals found in plants. Polyphenol compounds are able to inhibit oxidation reactions through radical scavenging by donating an electron to unpaired electrons in free radicals

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so that the number of free radicals is reduced (Shahidi and Zhong, 2015). The average value of total polyphenols can be seen in Table 3.

Table 3. Analysis Result of Total Polyphenols of Boiling Water of Ciplukan Plants

Parts of Plant	Total Polyphenol (mg GAE/g) ± Standard Deviasion		
	A (10 min)	B (20 min)	C (30 min)
Leaves	912,67 ± 66,75 a	970,67 ± 83,58 b	1210,5 ± 56,02 c
Fruits	368,67 ± 53,41 a	428,5 ± 41,39 b	446,83 ± 59,68 c
Stems	459,67 ± 108,05 a	639,67 ± 88,44 b	1020,67 ± 232,02 c
Roots	301,5 ± 33,68 a	543,5 ± 196,7 b	530,5 ± 70,04 c
Mixture *	337,17 ± 96,5 a	440,33 ± 54,91 b	371,67 ± 104,1 c
KK = 7,53			

Note : (±) Standard of Deviasion

The numbers on the same lane followed by unequal small letters are significantly different according to DNMRT at α = 5%.

The results of total polyphenol showed that each part of the ciplukan plant has a different content of total polyphenols. The highest total polyphenol was found leaves group, followed by the stem, root, fruit, and mixture groups. Cobaleda-Velasco (2017) in his research on the *Physalis ixocarpa* plant also said that the results of his study showed the variation of polyphenol component in each different plant part. The stem has the highest concentration of total polyphenol while the fruit has the lowest total polyphenol concentration.

The highest total polyphenol was obtained in leaves with treatment C with 30 min of boiling time of 1210.5 mg GAE / g. According to Cobaleda-Velasco (2017), in the stems and leaves there is chlorogenic acid which is one of polyphenols in ciplukan plants. Chlorogenic acid is an ester formed from trans-cinnamic acid and quinic acid having a hydroxyl group at an axial position on the carbon 1 and 3 and the equatorial hydroxyl of 4 and 5 carbon. According to a Cobaleda-velasco et al (2017) study, ciplukan leaf had several phenol components, which are: kaemferol-3,7-di-O-glycoside, quersetin-3-O-glycoside, and kaemferol-3-O -glycosides.

The highest total polyphenol in leaves, fruits, and stems were obtained at treatment C with boiling time of 30 min. This proved that the longer the boiling the total polyphenols became higher. In accordance with the principle the longer the extraction, the contact between the solvent and the material will be longer so that from both materials will occur mass deposition by diffusion until the balance of the solution concentration inside and outside the material extraction. In the root and mixture of plant parts the highest value was obtained at treatment B with 20 min of boiling time, and decreased at the next minute. The decreased in total polyphenols was thought to be due to the length of boiling time, the longer the heat received by the material to extract bioactive substances that lead to oxidation so as to reduced the content of phenol

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compounds that were vulnerable to heat. The high temperature and the length of boiling time can affect the content of a compound (Ajagun *et al.,* 2017).

In leaves and fruits groups, the highest antioxidant activity found in treatment C with boiling time of 30 min. This showed that the longer the boiling time the higher antioxidant obtained. This was in accordance with the principle that the longer the time of extraction, the contact between solvent and sample became longer so from both material occured a mass suspension by diffusion there was a balance of solution concentration inside and outside the extraction material. However, in the stem, roots, and mixtures of whole parts the highest antioxidant activity was obtained in treatment B with a boiling time of 20 min and decreased in the next minute. This was probably because natural antioxidants had different chemical structures and stability e.g., α-tocopherol is quite resistant to heat, loss during processing and largely due to the oxidation process. Carotenoids in plant cells in addition to being in a complex form with proteins also had multiple bond structures, so they are relatively stable against cooking but are very sensitive to oxidation (Mezzomo and Ferreira, 2016).

Table 4 showed the increased of antioxidant activity in the leaves and fruits groups, but there was a decreased in the stems, roots, and mixture groups after 20 min of boiling. This was also the same for the total polyphenols obtained except in the stems group which still had an increase of total polyphenols up to 30 min of boiling. An increased in antioxidant activity was persumably due to a relationship or correlation between the total polyphenol content (mg GAE / g sample) to antioxidant activity in the sample. The higher the content of polyphenols resulted the higher antioxidant activity. Same result, Abdul-Hafeez (2014) confirmed that the total phenolic content contributes 77% to antioxidant activity in plants.

Table 4. Analysis Result of Antioxidant Activity of Boiling Water of Ciplukan Plants

Parts of Plant	Aktivitas Antioksidan (%) ± Standard Deviasion		
	A (10 min)	B (20 min)	C (30 min)
Leaves	74,28 ± 10,06	84,01 ± 1,91	88,32 ± 0,59
Fruits	31,81 ± 6,78	32,14 ± 3,42	35,53 ± 5,54
Stems	53,51 ± 5,21	66,98 ± 8,09	55,19 ± 8,43
Roots	33,74 ± 3,66	38,22 ± 9,78	36,61 ± 5,54
Mixture *	49,46 ± 3,35	66,48 ± 12,5	49,84 ± 5,04
KK = 3,54			

Note : (±) Standard of Deviasion

Sensory Evaluation of Ciplukan Leaves Syrup

The sensory evaluation test aimed to determine the level of panelist's preference for the product that being tested. The product that tested was a syrup product made from ciplukan leaves boiling water which had the highest total polyphenol content and antioxidant activity. Panelists who tested consisted of 20 people

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with the specifications tested were color, aroma, and taste. The test used a hedonic scale test which had a very dislike range (numerical scale = 1) to very like (numerical scale = 5). The average results of organoleptic test of ciplukan lealves syrup can be seen in Table 5.

Tabel 5. Result of organoleptik test of ciplukan leaves syrup

Specifications	Value ± Standard Deviasion
Color	4,05 ± 0,61
Aroma	3,3 ± 0,57
Taste	2,65 ± 0,99

Based on the result of test given by panelists, it can be seen the panelist's favorite level to the color, aroma, and taste of the ciplukan leaves syrup product. The panelist's favorite level of color was on a scale of 4.05 (likes), 3.3 (regular) scents, and 2.65 flavors (dislike - usual). The color of ciplukan leaves syrup was a clear green like the bud of leaves. The fresh green color can increased the appetite to drink it. The smell of sugar from the syrup was not too strong and was not have a distinctive aroma. The flavor of this ciplukan leaves syrup was sweetly mixed with bitter. Sweet taste tasted at the beginning and then followed by a bitter taste. Its high content of polyphenols in the leaves make the syrup tasted bitter, but it gave health effects on the body.

In the study of Roopalatha and Nair (2013) said that the leaves of moringa and leaves of ciplukan was positive contain all secondary metabolite compounds tested including flavonoids, alkaloids, steroids, tannins, saponins, anthraquinones and terpenoids. The presence of these metabolite compounds cause moringa leaves and ciplukan leaves known as a nutritious medicinal plants today. Saponins contained in the leaves of ciplukan provide a bitter taste and coolant properties and efficacious as anti-tumor and inhibit the growth of cancer, especially colon cancer. The bitter taste made some panelists was not like ciplukan leaves syrup products.

CONCLUSION

Based on the results of research that has been done can be concluded that the different treatment of boiling time in each part of ciplukan plant significantly affect the total polyphenols, but no significant effect on the antioxidant activity obtained. Different group had a very significant effect on total polyphenols and antioxidant activity. Moreover, the best boiling time was treatment C (30 minutes boiling time) because it had the most optimal time efficiency in extracting bioactive components from ciplukan plants maximally.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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