Effect of ultrasonic assisted extraction on Dayak onion powder extraction (*Eleutherine palmifolia*)

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Abstract. Dayak onion (*Eleutherine palmifolia*) is a typical plant of Central Borneo, Indonesia, which contains flavonoids, saponins, polyphenols, alkaloids, glycosides, steroids, phenolic, tannins, triterpenoids, and quinones. It has bioactivity as an antioxidant. Extraction using the ultrasonic assisted extraction (UAE) method can increase phenol compounds and antioxidants in a shorter time. The purpose of this study was to determine the effect of variations in the amplitude and timing of sonication of purified water-based UAE to increase the value of antioxidant activity and the total value of phenol from Dayak onion powder extract. The amplitude variations used in this study were 20%, 35%, and 50%, and the variations of the sonication time used were 5, 10, and 15 minutes. Antioxidant activity was tested using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Total phenol content was tested by the Folin-Ciocalteu method. In the results of this study, the best value for antioxidant activity on Dayak onion powder extract was at a variation of 50% amplitude and 5-minute sonication time, which was 2.433 ± 0.283 mg/ml. While the best value on the test of total phenol of Dayak onion powder extract was at a variation of 35% amplitude and 5-minute sonication time, which was equal to 6.558 ± 1.68 mg GAE/g. Based on the results of ANOVA statistical analysis, it was found that there was a significant effect on increasing antioxidant activity with a significant value of 0.048 on variations in the combination of amplitude and sonication time.

1. Introduction

Indonesia is a country that has abundant biodiversity, one of which is a type of plant that is thought to have efficacy as a medicine. One plant that has many benefits for health is Dayak onion (*Eleutherine palmifolia*) [1]. Dayak Onion is a typical plant of Central Borneo, Indonesia. The development and utilization of dayak onion are still not extensive. However, the existence of this plant has been used by local people of Central Borneo as traditional medicine for breast cancer, colon cancer, hypertension, diabetes mellitus, cholesterol-lowering, boils, and stroke prevention [2]. Dayak Onion contains flavonoid compounds, saponins, polyphenols, alkaloids, glycosides, steroids, phenolics, tannins, triterpenoids, and quinones [3]. This plant contains naphtoquinone, which are known as antimicrobial, antifungal, antiviral, and antiparasitic. Besides, naphtoquinone have bioactivity as anticancer and antioxidants [4].

Antioxidants function to prevent or reduce the risk posed by free radical activity by donating one atom of protons so that free radicals can be stable and not reactive [5]. To get antioxidants from Dayak onion can be done by extraction. Ultrasonic Assisted Extraction (UAE) is one of the ultrasonic assisted extraction methods. Ultrasonic waves are sound waves that have frequencies above human hearing,
which is ≥ 20 kHz [6]. The main advantage of extraction with the help of ultrasonic waves is greater efficiency with a shorter operating time. The study of extraction using the UAE method has been carried out. Deng [7] was optimizing UAE for extracting total phenolic compounds (TPC) from fresh olives. The results showed that UAE could effectively increase the yield of phenolic compounds from olives. Wong [8] was also successfully optimizing UAE for extracting isoflavonoids from Puerariae Lobata with maximal bioactivity by using response surface methodology. Amiri [9] has proven the effectiveness of the UAE in extracting fatty acids from Aesculus hippocastanum fruit. Chen [10] has also proven the effectiveness of the UAE in extracting polysaccharides from Mentha haplocalyx. According to Baskar [11], the UAE can also be used effectively to extract bio-oil from Madhuca indica seeds to be used as biodiesel raw material. The purpose of this study was to determine the effect of variations in UAE amplitude and sonication time on the extraction results of total phenol compounds and antioxidant activity from Dayak onion powder. The extraction results with the UAE method will be compared with conventional extraction methods.

2. Materials and Method

This research was conducted at the Central Laboratory of Life Sciences, Universitas Brawijaya, Indonesia. The tools used in this study are as follows: 1) Ultrasonic Vibrating Horn (Sonic Type-450 Banson, USA) which was used to assist the extraction process with ultrasonic waves; 2) UV-Vis spectrophotometry (Thermo scientific Genesys UV 10, United States) which was used to test antioxidant activity and total phenol. The material of Dayak onion powder without treatment used in this study was obtained from the village of Tirtomirmo, Kasihan Subdistrict, Bantul Regency, Yogyakarta, Indonesia.

The extraction process of Dayak onion powder began with the dissolution process of 20 g of Dayak onion powder with 300 mL of distilled water solvent, which was then homogenized. The extraction process was done by using the UAE with a combination of treatment variations in amplitude of 20, 35, and 50% and variations in extraction time of 5, 10, and 15 minutes. Each treatment was carried out three times. Then the extraction was filtered with fine filter paper that produces the filtrate. The filtrate was then evaporated at a speed of 60 rpm, temperature of 50 °C, pressure of atmospheric pressure which produces Dayak onion extract. Then the next process was testing the yield value [12], total phenol [13, 14], and antioxidant activity [15, 16].

3. Results and Discussion

The characteristics of Dayak onion powder in the form of water content, total phenol, and antioxidant activity can be seen in Table 1. Based on Table 1, it can be seen that the water content of Dayak onion powder is 16.01%. The water content in food ingredients determines the freshness and durability of food. High water levels can cause bacteria, mold, and yeast to breed easily so that changes will occur in food. Phenol level for raw material for Dayak onion powder is 4.68%. IC50 of 11.78 mg/mL obtained the antioxidant activity of the raw material for Dayak onion powder. The IC50 value is thought to be influenced by phenol compounds, which have influence on antioxidant activity. In Dayak onions powder, there are compounds of polyphenols and flavonoids, which are often referred to as natural antioxidants, where both will work together to ward off free radicals. The content of phenols and flavonoids in Dayak onions will affect antioxidant activity and IC50 in the Dayak onion powder.

Table 1. The characteristics of Dayak onion powder

| No. | Parameter          | Value       |
|-----|--------------------|-------------|
| 1.  | Water content      | 16.01%      |
| 2.  | Total phenol       | 0.47 mg GAE/g |
| 3.  | Antioxidant activity | 11.78 mg/mL |

Yield is a parameter to find out the product results from a production process. Yield is an important parameter to find out whether the process on producing a product has a high economic value. The extract yield was calculated based on the ratio between the final mass (weight of the extract produced) and the
initial mass (weight of the dry matter) multiplied by 100%. The yield value obtained based on the extract can vary. The results of the extract of Dayak onion extract can be seen in Figure 1. In Figure 1, there is a fluctuating relationship in the yield produced. The difference in yield produced is caused by the difference in mass of extract produced. The greater the mass of the extract produced will increase the yield of Dayak onion powder. The difference in the yield of Dayak onion powder was also influenced by the sonication amplitude and the extraction time contacted on the sample material. The volume of solvent can also cause the high yield value. The more volume of solvent used, the higher the yield produced.

![Figure 1. Effect of extraction time on Dayak onion yield](image)

The total phenol content was tested using the Folin-Ciocalteu method. The highest total phenol value was in the 5th minute, which was 6.56 ± 1.68 mg GAE/g dry extract with an amplitude of 35%. While the lowest total phenol value was in the 10th minute, which was 4.66 ± 0.57 mg GAE/g dry extract with an amplitude of 50%. The standard deviation is obtained with a range of 0.57-2.08. The smaller the standard deviation value, the sample data obtained will be closer to the average value. Figure 2 is a graph of the effect of sonication time on total phenol. In Figure 2 shows that the sonication time does not have a significant impact on the total phenol produced in each sample, where the value of total phenol fluctuates. The increase in total phenol occurred in the 5th minute with an amplitude of 35%, but when the time was increased to 10 minutes with the same amplitude, there was a decrease in the total phenol produced. This is presumably because when the extraction lasted for 10 minutes with high amplitude, there was saturation in the material used. Long sonication time can make the solvent become saturated quickly and not able to extract the ingredients optimally so that it can reduce total phenol. In Figure 2 shows no effect of sonication time on total phenol, where the value of total phenol fluctuates. The fluctuating temperature causes the value of the total phenol to fluctuate. Besides, a decrease in the total phenol was due to the degradation of the compound in Dayak onion powder. In addition, high temperatures cause lower efficiency in the cavitation phenomenon [17]. Hashemi [18] states that total phenol increases when the temperature and length of sonication increase, but in cases with a temperature of 40 °C, a decrease in the total phenol value has been studied with increasing time. This corresponds to the data that has been obtained. Figure 3 is a comparison of the results of total phenol using the UAE with controls. Figure 3 shows that the total phenol produced by the UAE method is better than the maceration method, which is carried out for 24 hours.
Figure 2. Effect of extraction time on Dayak onion total phenol

Figure 3. Comparison of UAE's best results with control on total phenol

Figure 4 shows no effect on the length of sonication time on antioxidant activity, where antioxidant activity fluctuates. In the research on Dayak onion powder extraction using UAE, it shows a higher value of antioxidant activity (lowest value of IC50) with a shorter extraction time, which is 5 minutes with a high amplitude of 50%. The extraction time will also affect the results of the extraction itself, where the extraction time also causes contact between the material and the solvent to release more compounds in the material. The results of antioxidant activity from Dayak onion powder extract can be concluded to have weak antioxidant activity but still has the potential as an antioxidant. The treatment of extraction time that is too long will cause a stress reaction that has the potential to increase the cell defense mechanism by increasing the synthesis of secondary metabolites [19]. The effect of the cell membrane being destroyed can lead to permanent perforation and cause cell death. These dead cells can reduce the compounds contained in cells so that optimal sonication time is needed to get the best IC50 results. The IC50 value does not represent the magnitude of the antioxidant content in Dayak onion powder but only classifies the level of strength of antioxidant activity. Heat from the ultrasonic system is thought to affect the percent of inhibition value. The higher the amplitude, the higher the temperature. Excessive heat causes the extraction temperature to reach the labile point of the target compound and causes damage to the target compound thermally, so it is possible to increase the extract concentration, which is not too sharp, even decrease the extract until the material cannot be extracted again. The degradation of antioxidant compounds can be caused by oxidation reactions, i.e. the termination of covalent bonds and an increase in the rate of oxidation by heat. Antioxidant compounds that have been oxidized will be damaged and reduce their ability to reduce and counteract free radicals.
Figure 5 shows a comparison of the value of antioxidant activity between the UAE's best treatment (lowest value of IC50) and control. The antioxidant activity data obtained was analyzed by ANOVA with a confidence level of 5%. The analysis was carried out using the Turkey and Bonferroni method to analyze the real effect of the amplitude and sonication time on the extraction process of Dayak onion powder. Based on the result, it was found that the amplitude and sonication time of Dayak onion powder extraction had a significant effect with a confidence interval (α = 0.05) with a significant value of 0.048.

Determination of the best treatment was done by scoring method based on the level of importance. The importance level includes antioxidant activity (score = 1), total phenol (score = 0.9), and yield (score = 0.8), respectively. Therefore, the weight value for each parameter was antioxidant activity of 0.370, total phenol of 0.333, and yield of 0.296. From the weighting calculation, the best amplitude value was 35% with a sonication exposure time of 10 minutes.

**Figure 4.** Effect of UAE sonication time on IC50 values

**Figure 5.** Comparison of UAE's best results with control on IC50 values

4. Conclusions
UAE method in the extraction process can increase the antioxidant activity of Dayak onion powder extract. The best treatment in yield (weight of importance = 0.296), total phenol value (weight of importance = 0.333), and antioxidant activity (weight of importance = 0.370) of Dayak onion extract with UAE is at variation of 35% amplitude for 10 minutes sonication time. The optimal results obtained show a yield value of 34.67%, total phenol value of 6.17 ± 1.19 mg GAE/g, and antioxidant activity value of 2.64 ± 1.23 mg/mL. This value is better than the control sample without the UAE method, where the value of antioxidant activity is 6.55 ± 0.86 mg/mL, and the total phenol content is 3.41 ± 0.53 mg GAE/g. Based on the results of the ANOVA analysis with a confidence interval (α = 0.05), it was
found that there was a significant effect on increasing antioxidant activity with a significant value of 0.048 on amplitude and sonication time.

References
[1] Chabib L, Muhtadi W K, Rizki M I, Rahman R A, Suhendri M R, Hidayat A 2018 Potential medicinal plants for improve the immune system from Borneo Island and the prospect to be developed as nanomedicine MATEC Web Conferences ICET4SD 154 04006 1-6.
[2] Febrinda A E, Yuliana N D, Ridwan E, Wresdiyati T, Astawan M 2014 Hyperglycemic control and diabetes complication preventive activities of Bawang Dayak (Eleutherine palmifolia L. Merr.) bulbs extracts in alloxan-diabetic rats Int. Food Res. J. 21 4 1405-1411.
[3] Wicaksno I A, Firmansyah I, Indradi R B, Sulistiyaningsih S 2018 In vitro of Eleutherine palmifolia L. ethanolic extract against Bacillus cereus isolate J. Pharma. Sci. Res. 10 11 118-124.
[4] Kuntorini E M, Dewi M, Misrina 2016 Anatomical structure and antioxidant activity of red bulb plant (Eleutherine americana) on different plant age Biodiversitas 17 1 229-233.
[5] Wang L, Luo Y, Xia F, Wu Z 2018 Quickly verifying the antioxidant contribution of the individual composition in natural antioxidants by HPLC-free radical scavenging detection LWT 96 461-468.
[6] Rodsamran P, Sothornvit, R 2019 Extraction of phenolic compounds from lime peel waste using ultrasonic-assisted and microwave-assisted extractions Food Biosci. 28 66-73.
[7] Deng J, Xu Z, Xiang C, Liu J, Zhou L, Li T, Yang Z, Ding C 2017 Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives Ultrason. Sonochem. 37 328-334.
[8] Wong K H, Li G Q, Li K M, Naumovski V R, Chan K 2017 Optimisation of Pueraria isoflavonoids by response surface methodology using ultrasonic-assisted extraction Food Chem. 231 231-237.
[9] Amiri S, Shakeri A, Sohrabi M R, Khalajzadeh S, Ghasemi E 2019 Optimization of ultrasonic assisted extraction of fatty acids from Aesculus hippocastanum fruit by response surface methodology Food Chem. 271 762-766.
[10] Chen G, Fang C, Chen, X, Wang Z, Liu M, Kan J 2019 High-pressure ultrasonic-assisted extraction of polysaccharides from Mentha haplocalyx: structure, functional and biological activities Ind. Crops Prod. 130 273-284.
[11] Baskar G, Naveenkumar R, Mohanapriya N, Nivetha R, Aiswarya R 2018 Optimization and kinetics of ultrasonic assisted bioroil extraction from Madhuca indica seeds Ind. Crops Prod. 124 954-959.
[12] Ye J, Hua X, Wang M, Zhang W, Yang R 2019 Effect of extraction pH on the yield and physicochemical properties of polysaccharides extracts from peanut sediment of aqueous extraction process LWT 106 137-144.
[13] Singleton V L, Rossi J A 1965 Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents Am. J. Enology Viticul. 16 144-158.
[14] Kuck L S, Wesołowski C P Z 2017 Effect of temperature and relative humidity on stability following simulated gastro-intestinal digestion of microcapsules of Bordo grape skin phenolic extract produced with different carrier agents Food Chem. 230 257-264.
[15] Miliauskas G, Venkutonis P R, Van Beek T A 2004 Screening of radical scavenging activity of some medicinal and aromatic plant extracts Food Chem. 85 231-237.
[16] Arina M Z L, Harisun Y 2019 Effect of extraction temperatures on tannin content and antioxidant activity of Quercus infectoria (Manjakani) Biocatal. Agric. Biotechnol. 19 101-104.
[17] Yeong Y L, Pang S F, Chong S Y, Gim bun J 2018 Comparison of microwave and ultrasonic assisted extraction of kaempferol from Cassia alata Int. J. Eng. Technol. 7 84-89.
[18] Hashemi S M B, Ghorashi S, Hadizadeh F, Zarei Z, Yazdani M, Noormohammadi M 2017 Effect of amplitude of ultrasound-assisted solvent extraction and extraction temperature on the kinetics, thermodynamics, antioxidant and antimicrobial activity J. Agric. Sci. Technol. 9 1517-1526.
[19] Korma S A, Alahmad K, Ali A H, Shoaib M, Abed S M, Yves H, Atindana J N, Qin J 2016 Application of pulsed electric field technology in apple juice processing *Austin J. Food Sci.* **4** 2 1-5.