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Effects of Exposure to Ultrasonic Waves on Extraction of Evaporation Methods on The Anthocyanin Content of Green Betal and Basil Leaves

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Abstract: Synthetic anticancer agents have serious side effects on health. Therefore, it is necessary to develop natural anticancer agents with better effectiveness for cancer treatment. This study aims to determine the effect of exposure to ultrasonic waves on the extraction process by evaporation method on grain temperature, yield, and anthocyanin content of green betel and basil leaf. Exposure was carried out using 120 W ultrasonic waves for 0-30 minutes. The results showed that exposure for 30 minutes increased the temperature of the green betel leaf and basil leaves from 27°C to 52°C. Extraction by exposure to ultrasonic waves for 10 minutes obtained the highest yields of 13.87±1.10 % and 18.73±3.74% for green betel and basil leaf, respectively. Extraction with ultrasonic wave exposure time of 25 minutes resulted in the highest anthocyanin content, namely 490.10±6.67 mg/L and 242.65±9.09 mg/L for green betel and basil leaf, respectively. The high anthocyanin content is influenced by the exposure time and the temperature of the extracted granules. The time of exposure to ultrasonic waves of more than 25 minutes makes the grain temperature more than 50°C, so that the anthocyanin content of the extraction results decreases.

Keywords: Anthocyanin; Basil; Betel; Content; Leaf; Yield

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Introduction

Cancer is a severe health problem worldwide (Liu et al., 2014) and, it is estimated that there will be 19.3 million new cancer cases and nearly 10.0 million cancer deaths by 2020 (Sung et al., 2021). Chemotherapy is the most commonly used cancer therapy method because it is the most effective approach (Hosseinzadeh et al., 2017; Shi et al., 2014). However, this method produces serious side effects on human health due to synthetic antitumor agents (Liu et al., 2014). To overcome this problem, it is necessary to develop natural products with better effectiveness for cancer treatment (Sun et al., 2017).

In recent years, anthocyanins isolated from natural sources have been shown to have significant health benefits related to antioxidant, anti-inflammatory, and anticancer activities (de Sousa Moraes et al., 2019). Previous studies have shown that anthocyanins are potential agents for preventing and treating cancer (Joshi & Goyal, 2011). Therefore, searching for new and safe anthocyanins from organic sources is increasingly important. Anthocyanin compounds are generally found in plants with natural antioxidant activity that can capture free radical molecules (Wu et al., 2018; Tena et al., 2020). Basil leaves (*Ocimum basilicum L*) and green betel leaf (*Piper betle Linn*) are plants that have natural antioxidant activity compounds and are widely available in Indonesia.

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Anthocyanin compounds from green betel and basil leaf can be obtained by extraction. In general, extraction can be done by evaporation, maceration, percolation, reflux, and others. Conventional extraction has the disadvantage that it takes a long time, has high extraction temperature, and low extract yield, but has high energy consumption (Xinsheng Wang X et al, 2012). One effort to overcome this is to add exposure to ultrasonic waves in the extraction process. The mechanical effect of ultrasonic waves is to increase the penetration of the liquid into the cell membrane wall, support the release of cell components and increase mass transfer (Keil, 2007). Exposure to ultrasonic waves can cause a cavitation effect that can break down cell walls. The bioactive components come out easily and obtain maximum extract results with a much shorter extraction process (Mellenthin, 2002).

Previous studies reported that ultrasonic wave-assisted extraction has been successful and widely used because of its high efficiency, time-saving, and environmental friendliness (Wang J. et al., 2008; Wang L. and Weller, 2006). Compared to other extraction methods, ultrasonic wave-assisted extraction is much more efficient, less solvent, and time-saving (Tao et al., 2014). Previous studies have also reported that when aqueous solutions are irradiated with ultrasound, the H-O bonds in water are cleaved to form hydroxyl radicals and hydrogen atoms (Bremner et al., 2010). This process results from cavitation, where extremely high temperatures and pressures are generated in the bursting bubbles (Bremner et al., 2010). Ultrasonography with a frequency of 20 kHz can instantly generate local high temperatures, high pressures, and super-speed jet streams (Jiang et al., 2014) (Monnier et al., 1999). Ultrasonic treatment can increase the contact between the material and the solvent and speed up the reaction (Ma et al., 2021).

Based on previous research reports, exposure to ultrasonic waves on wet organic material causes cavitation, which will break the cell and increase the local temperature of the material. The local temperature will conduct to the surrounding area, where the speed of conduction depends on the material's conductivity. The longer the exposure to ultrasonic waves is given, the higher the temperature of the material. Therefore, prolonged exposure to ultrasonic waves will damage the material; as a result, it will reduce the levels of anthocyanins produced. Exposure to ultrasonic waves with excessive power and time will accelerate the decomposition of the resulting material or change the product's physical properties, which is not conducive to the reaction (Klanian G. & Preciat T, 2017). The novelty of this article is to observe the effect of time exposure to ultrasonic waves on the temperature of the material and its impact on yield and anthocyanin content so that the optimum point is obtained.

This study aims to explain the effect of time exposure to ultrasonic waves on the increase in material temperature, yield, and anthocyanin contens produced.

Method

Sample Preparation

The research samples were fresh green betel leaves (*Piper betel Linn*) and basil leaves (*Ocimum basilicum L*). Next, the green betel leaves and basil leaves were dried at 50^{o}C for 15 hours in an oven. The basil and betel leaves are mashed using a blender and then sieved with a 100 mesh sieve in warm conditions

Extraction process

Weigh the powder of green betel and basil leaf each weighing 5 grams. Leaf powder that has been weighed is put into a glass beaker. Next, pour 50 mL of 96% ethanol solution into a glass beaker that already contains green betel leaf or basil leaf powder. The mixture of leaves and ethanol was stirred until homogeneous and then put into the Erlenmeyer. The leaf powder and ethanol mixture in the elemeyer was exposed to ultrasonic waves for 0.0-30.0 minutes, the ultrasonic wave power was 120 W and the frequency was 40 kHz. Ultrasonic waves are generated from Skymen Cleaning JP-020. After evaporating the mixture of leaf powder and ethanol using a rotary evaporator at a pressure of 125 mbar and a temperature of 40^{o}C. The flow of the research implementation is as shown in Figure 1.

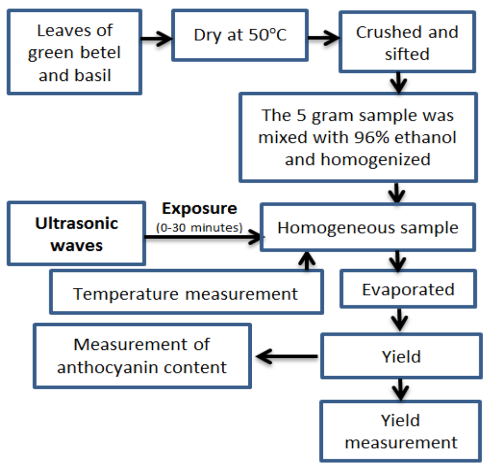


Figure 1. Schematic of research implementation steps

Measurement

The sample temperature measurement was carried out using Baumer Temperature Gauges CB. Measurement of yield is done by weighing the initial weight of the sample just before being exposed to ultrasonic waves and the final weight after the evaporation process. The amount of yield is calculated by the equation 1

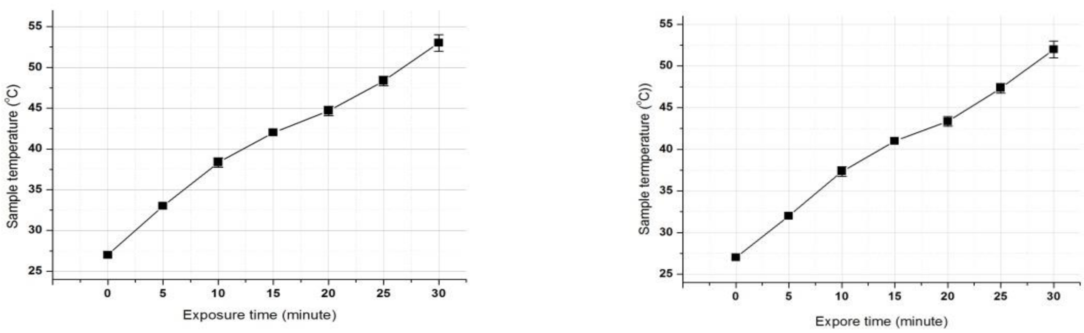
$$x = \frac{\text{Final mass}}{\text{Initial mass}} x\ 100\% \quad \text{..........................} \ (1)$$

Measurement of anthocyanin content was carried out using a UV-Vis spectrometer at a wavelength of 510 nm.

Result and Discussion

Sample Temperature Change

The interaction between ultrasonic waves with the material causes an increase in the temperature of the material. Figure 2a. is the effect of time of exposure to ultrasonic waves on the temperature of the betel leaf grain. Betel leaf grain temperature increases linearly with increasing exposure time. Before exposure, the temperature of the betel leaf is 27°C, and after 30 minutes of exposure, it becomes 52°C. Figure 2b. is the effect of time exposure to ultrasonic waves on the temperature of the basil leaf granules. The temperature of basil leaves increases with increasing exposure time. Before compression, the temperature of the basil leaves was 27°C, and the temperature for 30 minutes was 52°C. Identical conditions have been presented previously that therapeutic ultrasound in continuous mode with frequencies of 1 MHz and 3 MHz and intensities of 1 W/cm^2 and 2 W/cm^2 for 2 minutes causes heating of metal plates and adjacent structures (Andrades et al., 2014). Heating is caused by the absorption of ultrasonic wave energy being more significant than the released (O'Brien, 2007). Heat production depends on acoustic impedance and tissue absorption (Radzi & Zaiki, 2018).

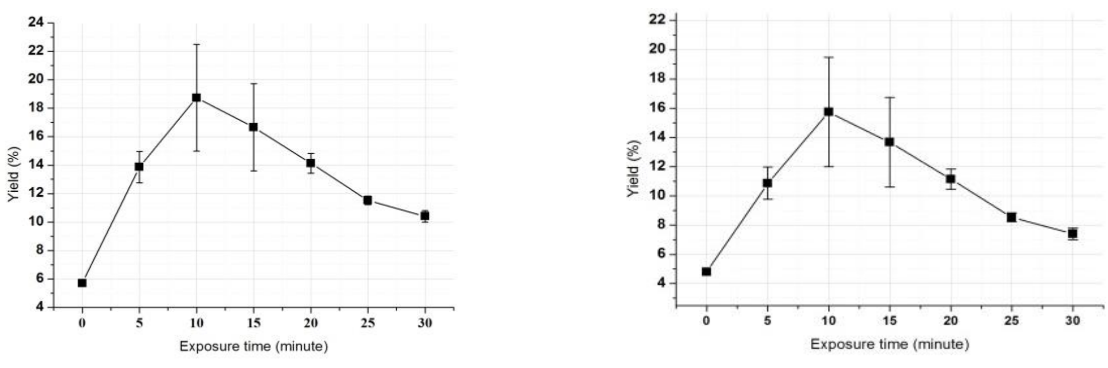


(a) (b)

Figure 2. Effect of time exposure to ultrasonic waves on grain temperature (a) green betel leaf, (b) basil leaf.

Yield

The addition of exposure to ultrasonic waves in the extraction process using evaporation affects the yield produced. Figure 3a shows that the exposure time of 5 and 10 minutes made the yield of betel leaf significantly increased from 5.7±0% to 13.87±1.10 % and 18.73±3.74%, respectively. Exposure for 15-30 minutes of yield started to decrease and exposure for 30 minutes yielded yield was 10.4 ± 0.4%. Figure 3b shows the effect of time exposure to ultrasonic waves on the yield produced from basil leaves. The graph shows that exposure for 5 and 10 minutes of yield increased significantly, from 4.8±0% to 10,87±1.10% and 15.73±3.74%, respectively. Exposure to ultrasonic waves from 15-30 minutes decreased the yield and became 7.4±0.4% at exposure for 30 minutes. The highest yield was obtained by exposure to ultrasonic waves for 10 minutes.

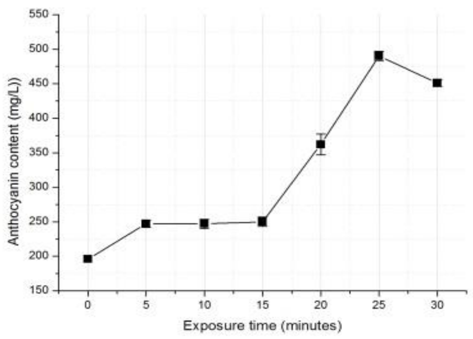


(a) (b)

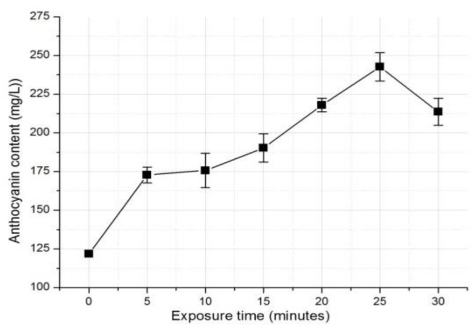
Figure 3. Effect of time exposure to ultrasonic waves on the yield of the extracted (a) green betel leaf, (b) basil leaf.

Anthocyanin content

The addition of exposure to ultrasonic waves in the extraction process using evaporation affects the anthocyanin content produced. Figure 4a shows a graph of the effect of exposure time on the anthocyanin content of betel leaf extract. The graph shows that exposure to ultrasonic waves for 25 minutes produced an extract with the highest anthocyanin content, namely 490.10±6.67 mg/L. The same condition occurred in the extraction of basil leaves, where without exposure, the anthocyanin content was 121.83±0 mg/L, and after exposure for 25, anthocyanin content was 242.65±9.09 mg/L, as shown in Figure 4b.



(a)



(b)

Figure 4. Effect of time exposure to ultrasonic waves on the anthocyanin content (a) green betel leaf, (b) basil leaf.

The anthocyanin content extracted with an exposure time of 0-10 minutes is relatively low because the betel leaf and basil leaf particles are smaller and have not undergone evaporation, so many compounds are also extracted. The increase in anthocyanin content occurs at an exposure time of 15-25 minutes because some elements with a boiling point below 50°C experience evaporation, while the anthocyanin compounds have not evaporated. Meanwhile, the low anthocyanin content at the exposure time of 30 minutes was due to some of the anthocyanin compounds undergoing evaporation.

Discussion

Exposure to ultrasonic waves affects the yield and content of anthocyanins produced from green betel and basil leaf extraction. The optimum yield obtained from exposure to ultrasonic waves for 10 minutes was 18.73±3.74% for betel leaf and 15.73±3.74% for basil leaves. The optimum anthocyanin content obtained from granules exposed to ultrasonic waves for 25 minutes was 490.10±6.67 mg/L for betel leaves and 242.65±9.09 mg/L for basil leaves.

An identical study using a microwave combined with a rotary evaporator obtained 33.7% basil leaves yield with an extraction temperature of 40°C and 6 minutes (Qorriaina et al., 2015). Extraction of betel leaf by immersion obtained the highest yield of 8.15% with an immersion time of 76 hours (Handoyo, 2020). Sancang wood extraction with a rotary evaporator obtained flavonoid content of 6.02% ± 0.6 and anthocyanin content of 2.43% ± 0.5 (Nomer et al., 2019). Exposure to microwaves in the extraction process using a rotary evaporator resulted in a larger yield than ultrasonic waves, however, the research conducted by Qorriaina et al has not reported the anthocyanin content produced. In this study, high yields resulted in low anthocyanin content.

Exposure to ultrasonic waves causes the temperature of betel leaf and basil leaves to increase. Exposure for 30 minutes made the temperature of the green betel leaf grains change from 27°C to 53°C and basil leaves from 27°C to 52°C. Exposure to ultrasonic waves causes cavitation of basil and basil leaves, making the green betel and basil leaf particles smaller. The smaller the granules make the extraction easier and faster. The cavitation process can cause an increase in the temperature of the particles, causing some substances to evaporate. Therefore, the anthocyanin content produced is higher. The longer the heating, the more degraded anthocyanins (Nayak et al., 2011), so that exposure for 30 minutes reduces the anthocyanin content. Anthocyanin degradation also occurs at higher temperatures (Muche et al., 2018). Therefore, exposure for 30 minutes made the grain temperature rise to 52°C, resulting in decreased anthocyanin content.

Conclusion

Ultrasonic wave exposure affects yield and anthocyanin content extracted using the evaporation method. Exposure for 10 minutes resulted in high yields but low anthocyanin contents. Exposure to ultrasonic waves for 25 minutes resulted in low yields, but high anthocyanin contents. Therefore, to obtain a high anthocyanin content, exposure to ultrasonic waves with a power of 120 W for 25 minutes is recommended.

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