The effect of solvent volume ratio and extraction time on the yield of red dye from sappanwood

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Abstract. Natural red dye from sappanwood (Caesalpinia sappan L) contains brazilin that can be used as an antioxidant source. The dye can be isolated by extraction using a polar solvent such as distilled water. The more water was added, the more extract can be obtained. However, the process cannot be straightforward since the excessive solvent consumes more energy cost in the extract product purification. The study aims to investigate the effect of water to sappanwood ratio and operational time on the effective time for the extraction of red dye from sappanwood assisted by ultrasound vibration. Here, the water to sappanwood ratios were varied from 5:1 to 8:1 at operational temperatures 60°C. With more excessive solvent, the yield of sappanwood extract increased. For example, at 60°C and solvent to sappanwood ratio 8:1, the process yielded 4.0% extract for 15 minutes. Compared to the literature with conventional extraction without ultrasound, this achievement was twofold. Lowering solvent to sappanwood ratio reduced the extract yield, but it will ease in product separation. Furthermore, extending extraction time increased the yield, but after 15 minutes the effect was limited.

1. Introduction

The visual appeal, especially color, is recognized as the first quality attribute assessed by consumers when selecting food and beverage products [1]. Color plays a role as the most essential element that may influence consumers’ perception and their purchasing decision [2,3]. Dyes are often added in order to maintain and enhance the color of processed food, improve flavour expectation, and provide more appetizing and appealing look that will lead to consumers’ acceptability. There are two types of dyes available on the market, natural and synthetic. Synthetic dyes are frequently used by food manufacturers because of their low cost, excellent coloring ability, and high pigment color stability [4]. However, the use of synthetic colors, at some points, poses potential health implications, such as cancer, allergic effect, hyperactivity, thyroids, and asthma [5]. These health concerns have raised public awareness towards natural coloring agents which is viewed as non-toxic, safe, biocompatible, biodegradable, and green colorant derived from natural resources [6]. Consequently, there are growing interest and demand on natural dyes over synthetic dyes which encourages many researchers worldwide to discover ideal technique and advancement in natural colorant technology.

The majority of natural dyes are originated from plant sources. Numerous parts of plants including roots, seeds, flowers, fruits, leaves, barks, and stems that contain pigments such as flavonoids, tannins, anthocyanins, carotenoids, quinones, etc. can be extracted into natural dyes [6]. One of the plants which contain natural red pigments is sappanwood (Caesalpinia sappan L.). It belongs to the Leguminosae family plant and widespread throughout the Southeast Asia region [7]. The heartwood of this plant is...
rich in brazilein, the most biologically active compound in sappanwood that can be transformed into red dyestuff, brazilein, under oxidation event. Brazilein is reported to have various pharmacological effects, such as antioxidant activity, antibacterial activity, anti-acne activity, anti-inflammatory activity, and antihyperglycemic activity [7,8]. The antioxidant source was evaluated for its capacity to inhibit free radical 1,1-diphenyl-2-picyrylhydrazyl (DPPH), brazilein exhibited higher antioxidant activity with IC50 value 57.2 µM compared to other antioxidant sources such as standard vitamin E and other fractions isolated from sappanwood such as brazilein, sappanchalcone, protosappanin B and C, confirming that natural dyes containing brazilein are considered potential natural antioxidant source that contains many health benefits [9].

Various extraction methods could be applied to recover natural dyes from plants depending on the polarity and thermal stability of the target compounds, including conventional extraction methods such as soxhlet, maceration, decoction, heating under reflux, and percolation, along with the latest innovative extraction methods, for instance, ultrasound-assisted extraction, microwave-assisted extraction, supercritical-fluid extraction and pressurized-liquid extraction [6]. Owing to the fact that the extraction method is crucial in determining effective and efficient results with minimum processing cost, the proper extraction method should be carefully chosen [10]. Ultrasound-assisted extraction (UAE) is considered superior among all previously mentioned methods. UAE is an inexpensive method that offers a simple operating procedure that requires only an ultrasonic bath and is able to reduce the time, solvent, temperature, and energy needed for the extraction process making it suitable for the extraction of chemically and thermally unstable compounds [11]. In addition, the ultrasound apparatus is easier and more affordable compared to other modern extraction methods such as supercritical fluid extraction and microwave-assisted extraction [12]. A study on the extraction of carvone and limonene from caraway seeds reported that UAE was found to be 1.3-2 times faster than a conventional method which substantiated the efficiency of UAE [13]. The application of UAE has also been successfully conducted in the extraction of natural dyes from beetroot with an enhancement in the extraction efficiency compared with static/magnetic stirring at 45°C [14]. Furthermore, compared to conventional soxhlet and maceration extraction, UAE has been proven to obviously improve extraction yield and ensure the reliability of the extract obtained based on its antioxidant activity examinations [15]. The ultrasound waves aid the rapid releasing of the targeted compounds from the plant materials to the solvents and increase the mass transfer rates by inducing the formation and explosion of cavitation bubbles, known as the cavitation effect, which cause the collision between bubbles and surfaces of plant material leading to cell disruption [16,17]. The SEM micrographs provided evidence that cell walls were apparently damaged by the use of ultrasound waves [13].

Different solvents could be used to perform the extraction of natural dyes assisted by ultrasound waves including water, acetone, ethyl acetate, and methanol [18]. Concerning health and environmental aspects, the use of water is preferable instead of organic solvents since organic solvents may have serious environmental and health issues [19,20]. Moreover, water is a polar solvent that sustains green processing as it produces zero pollution to the environment, besides having enormous advantages like inexpensive, non-poisonous, non-combustible, abundant in nature, eco-friendly, and helpful in facilitating the separation process of the mixture [21].

Several process parameters such as temperature, time, and the ratio of solvent to material are key factors influencing the performance of the UAE. Generally, the extraction yield will increase as the temperature, the time, and the solvent to material ratio increase. However, raising extraction temperature could promote the decomposition of the heat-sensitive compounds as well as antioxidant capacity in the plant material even though the higher temperature could accelerate the reaction process by inducing higher transfer mass [22,23]. Meanwhile, long extraction time is unnecessary in the UAE system because in most cases, the maximum extraction rate is obtained in just a few minutes, and over-processing could result in the degradation of biologically active compounds [24,25]. Related to the solvent to material ratio, the extraction yield usually increases with the addition of solvent volume. Although the effect of process parameters on ultrasound-assisted extraction was extensively investigated, to our knowledge, no report works on the effect of solvent volume ratio correlated with the...
extraction time on the yield of red dye from sappanwood have been conducted yet. This study, therefore, aims to investigate the effect of water to sappanwood ratio and operational time on the effective time for the extraction of red dye from sappanwood assisted by ultrasound vibration, hence the successful extraction of natural red dyes from the heartwood of sappanwood could be achieved.

2. Materials and Methods

2.1. Materials
The sappanwood (Caesalpinia sappan L.) heartwood powders were purchased from a local Herbal Market located in Yogyakarta, Indonesia. Initially, the powders were passed through 80 and 100 mesh sieves to select heartwood particles with an average diameter of 0.1635 mm in order to enhance the active surface area of sappanwood heartwood powders for maximum contact to the solvent used. The moisture content of the powders was measured at about 2.14%. The powders were stored in a sealed plastic bag at 4°C until subjected to ultrasound-assisted extraction (UAE) experiments with distilled water as a solvent. The reason for the use of distilled water as a solvent was because water is edible, polar, and capable to dissolve natural dye extracts from sappanwood.

2.2. Methods: Extraction Procedure
The experiment was performed with an ultrasonic bath (BUC 65L, B-One Ultrasonic Cleaner, China) at a constant temperature of 60°C. Approximately 5 g of dried sappanwood heartwood powders were transferred into a vial bottle (100 ml) with distilled water as the extracting solvent. The prepared samples were then placed in an ultrasonic bath for the extraction experiment. The parametric study was carried out in order to identify the effect of four different water to sappanwood ratios (5:1, 6:1, 7:1, 8:1 ml/g). The extraction conditions were set as follows: ultrasound frequency was 40 kHz, the extraction time was 5, 10, 15, 20, and 25 min, and the temperature was 60°C. After sonication, the samples were cooled at room temperature before being filtered through a filter paper using a Buchner funnel connected on a filtering flask with a side tube connected to a vacuum pump. The filtrates were then dried in an electric oven at 105°C according to gravimetric analysis until constant weight. The total yield of natural dye extracts after the drying process was calculated using equation 1.

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\text{Yield (\%)} = \frac{\text{weight of the extract after drying}}{\text{weight of the dry sample}} \times 100\%
\]  

(1)

3. Result and Discussion
The solvent to material ratio is an influential factor determining the mass transfer in the extraction process. Basically, the use of a larger liquid-solid ratio provides an increase in the concentration gradient between the surface and the interior of the target compound [17]. Based on this fact, it can be concluded that the greater the ratio of the solvent to the material, the more solvent penetrated into the material, thereby enlarging the contact surface. A larger solvent ratio has also an impact on reducing the saturation level of the solvent, hence improves the extraction yield as the components of the material can be extracted thoroughly.

In this study, the influence of solvent to material ratio parameter on the extraction yield of natural dyes from the heartwood of sappanwood was investigated. The experiment was carried out by the ultrasound-assisted extraction method at 60°C using distilled water as the extraction solvents. In this work, the water to sappanwood (WS) ratio was varied from 5:1 ml/g to 8:1 ml/g, respectively, and was extracted for 5 min, 10 min, 15 min, 20 min, and 25 min.
Figure 1. The influence of water to sappanwood ratio and extraction time on the yield of sappanwood natural dye extract, at 60°C.

Figure 1 presented the effect of different water to sappanwood ratios over the extraction yield. It was observed from the results that as the WS ratio increased from 5:1 to 6:1 ml/g, the extraction yield was also increased. The same tendency was seen to occur with the further addition of distilled water until the WS ratio reached 8:1 ml/g. The possible explanation for these findings is that the increasing of WS ratio from 5:1 ml/g to 8:1 ml/g provides higher concentration difference between the external solvent and the internal material cell wall, thus increases the rate of dissolution and accelerates the mass transfer process resulting in higher extraction yield [26]. Otherwise, in smaller water to sappanwood ratio, lower extraction yield was observed because there is insufficient solvent to support the transfer process of all extracts from the material matrix [12]. Nonetheless, the driving force would not be affected that much by the use of excessive solvent according to mass transfer limitations that tend to depend on solid interior [27]. Taking all these factors into consideration, at water to sappanwood ratio of 8:1 ml/g and 15 min of extraction time was considered as a good option for this present extraction process where around 4.0% of the extract yield was obtained. In comparison with a study on sappanwood extraction using a conventional method from literature, the achievement was twofold [28]. This enhancement has assured that the efficiency of ultrasound-assisted extraction is more eminent than the conventional one. The ability of ultrasound to improve the extraction yield is attributed to a cavitation phenomenon, a mechanism caused by high-intensity and high-frequency ultrasound waves that involve the formation and implosion of cavitation bubbles [10]. The collapse of bubbles generates local heating and high pressure, shock waves, and shear force resulting in cell wall disruption which allowed solvent to intensively penetrate into cells and facilitate the release of extractable compounds, thereby increase mass transfer rate and extraction efficiency [29,30].

4. Conclusion
The present study showed that the efficacy of ultrasound-assisted extraction of natural red dyes from the heartwood of sappanwood is significantly influenced by water to sappanwood ratio and sonication time. Higher water to sappanwood and extraction time was more efficient for extracting natural red dye from sappanwood. In addition, increasing the water to sappanwood ratio led to an increase in the extraction yield. Longer extraction time in water also increased extraction yield, but it must be noted that long extraction time may damage the target compounds. However, by using ultrasound-assisted extraction technique, a time-consuming extraction process could be avoided. The results of this study confirmed that ultrasound-assisted extraction (UAE) is suitable for the recovery of natural red dyes from the
heartwood of sappanwood and could be considered to be applied in a large-scale extraction as an interesting green technology which requires simpler, safer, and cheaper procedures.

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