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Ultrasound assisted extraction of sappan wood (*Caesalpinia sappan* L.) using different solvents

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Abstract

Caesalpinia sappan L. or known as sappan wood is a plant that had been used in traditional food, beverages and traditional medicine. It contains many active phytochemicals compound such as brazilin, sappanchalcone, xanthone, coumarin and flavones. Extraction is the common method to get the active compounds in plants and the use of new method of extraction is very recommended. The research was aimed to analyze the effect of different solvent (ethanol, water and 2-propanol) to the characteristic of sappan wood extract obtained from ultrasound assisted extraction (UAE) process. Approximately 10 g of sappan wood was mixed with 100 ml solvent and extracted using probe type of ultrasound extractor for 15 mins and 50^{0}C. Extracts were analyzed for their yield, color, phytochemical compound, total phenolic content (TPC) and antioxidant activity. Ethanol gives the highest yield (6.125%) with the color tends to be between ruby red and traffic red. This result is in accordance with the calculation of Hansen solubility parameter (HSP). All of the three extracts contained saponin, tannin, flavonoid and triterpenoid. Ethanol also gives the highest TPC (254.14 mg GAE/100 g) and lowest IC_{50} (3.19). It can be concluded that ethanol is better solvent to extract phytochemical component in sappan wood.

1. Introduction

Caesalpinia sappan L. or known as sappan wood is a plant that belongs to Leguminosae family and had been used in traditional food, beverages and traditional medicine (Nirmal *et al*., 2015; Settharaksa *et al*., 2019). Several phenolic compounds were found in sappan wood, including brazilin, sappanchalcone, xanthone, coumarin and flavones. Brazilin (Figure 1) is an active compound found in sappan wood and was reported to have several activities such as an antioxidant, antibacterial, antiallergic and anti-inflammatory (Nirmal *et al*., 2015; Rahayuningsih *et al*., 2018). Brazilin is easily oxidized to form brazilein, and this compound is responsible for the yellow-red color source.

Extraction is the common method to get the active compounds in plants. There are several extraction methods including maceration, soxhlet extraction and percolation. Those methods were considered as the conventional methods that has many drawbacks, such as longer time needed, more solvent used and low yield resulted. To overcome those drawbacks, the use of new method of extraction is very recommended (Sánchez-Camargo *et al*., 2020). Ultrasound assisted extraction (UAE) is new extraction method that use the ultrasonic wave to help the extraction process. This method has advantages such as short extraction time, the use of low temperature process with the high yield resulted, eco-friendly and also has low risk (Alfieri *et al*., 2022).

In the extraction process, the choice of solvent is one important point, since solvent will affect the chemical composition, functional properties of extract (Jacotet-

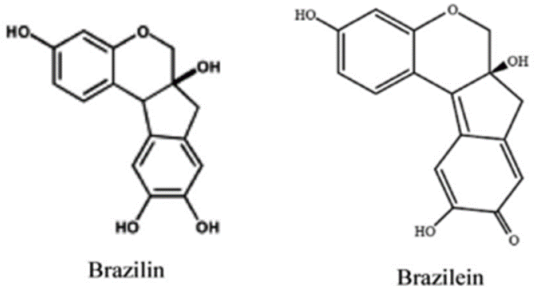

Figure 1. Chemical structure of brazilin and brazilein (Nirmal *et al.*, 2015).

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Navarro *et al*., 2018; Wakeel *et al*., 2019) and also efficiency of the compounds extracted (Sánchez-Camargo *et al*., 2020). Generally, solvent selection will depend on the solubility of solute in solvent. Solvent selected should fulfill the principle of "like dissolves like" with the target compound. Hansen solubility parameter (HSP) is one method to estimate the "closeness" of the solvent with the target compound. This method is derived from Hildebrand parameter that has been improved to overcome when the solvents used are polar and have hydrogen bonding. Total energy of vaporization in Hildebrand equation was described in three individual energies, they are atomic dispersion forces (δ_D), permanent dipole forces (δ_P), and hydrogen bonding (δ_H). Total energy density (δ_t) is then calculated using Equation (1) and the mutual solubility between solute and solvent is defined by R_a (Equation 2).

$$\delta_t^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \quad (1)$$

$$R_a = \sqrt{4\left(\delta_{Di} - \delta_{Dj}\right)^2 + \left(\delta_{Pi} - \delta_{Pj}\right)^2 + \left(\delta_{Hi} - \delta_{Hj}\right)^2} \quad (2)$$

Where R_a is the ratio of radius interaction, δ_t is the total energy density, δ_D is the atomic dispersion forces, δ_P is the dipole forces, δ_H is the hydrogen bonding, i is for solute and j is for solvent. Smaller R_a means that solvent is potential for extracting the solute since solvent and solute has greater affinity (Savova and Kolusheva, 2007).

Calculation of solvent solubility through HSP need to be compared with the experimental step to evaluate the result. Therefore, this research was aimed to investigate the effect of different solvent (ethanol, water and 2-propanol) on the characteristic of sappan wood extract obtained from UAE.

2. Materials and methods

2.1 Materials

Sappan wood was obtained from Ungaran area, Central Java, Indonesia. The whole part of the wood was used, including sapwood and heartwood. Three solvents were used, they are 96% ethanol (Bratachem), water/ aquadest (UD. Mitra Karya) and technical grade of 2-propanol (supplier of Indrasari). Reagent 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Folin-Ciocalteu's phenol reagent and gallic acid were purchased from Sigma Aldrich, while methanol and sodium carbonate were from Merck.

2.2 Extraction process

The sappan wood was sieved and grounded until passed 20 mess screening. Ten grams of sappan wood was mixed with 100 ml solvent and extracted using ultrasound extractor probe type (Ultrasonic Cell Disruptor TUE-500 with frequency 20-30 kHz and 12 mm of probe diameter) for 15 min and 50^0C. After extraction, solution was filtered using vacuum pump and solvent was separated using rotary evaporator (Heidolph, Hei-VAP Advantage) to get the extract. The yield of the extract was calculated using the Equation (3).

$$\text{Yield} = \frac{\text{mass of extract gained}}{\text{mass of raw material used}} \times 100\% \quad (3)$$

2.3 Color analysis

The color of extract solution was analyzed visually using the Classic RAL System for Color standard (Figure 2). The color of the solution was compared with the color standard, while pH was checked using pen type pH meter (PH-009 (I)).

Figure 2. Classic RAL system for color standard (Rahayuningsih *et al.*, 2018).

2.4 Phytochemical compounds analysis

Extracts were analyzed its phytochemical compounds qualitatively. The analysis included alkaloid (Warner method), triperpenoid and steroid (Lieberman Burchard method), flavonoid (Wilstater method), tanin ($FeCl_3$ 1% method) and saponin (Forth method).

2.5 Total phenolic content analysis

Total phenolic content of extract was analyzed using Folin-Ciocalteu's method (Arsiningtyas, 2021) with slight modification. Extract (0.1 g) was added with methanol until 10 mL. Then, 0.2 mL solution was taken and add with aquadest and 1 mL Folin-Ciocalteau's reagent. The solution was shaken and add with 3 ml 20% Na_2CO_3. Solution was incubated for 2 hrs in room temperature. Absorbance was analyzed using UV-Vis spectrophotometer (Genesis 10S) at 765 nm. Gallic acid was used as the standard for calibration curve.

2.6 Antioxidant activity

Antioxidant was measured using DPPH free radical scavenging activity. For this analysis, 1 mL sample was added with 4 ml 0.05 mM DPPH in methanol. Solution was incubated in dark place for 30 mins. Absorbance was measured at 515 nm using UV-Vis spectrophotometer. The inhibition was calculated using Equation (4).

$$\text{Inhibition (\%)} = \frac{A_0 - A_s}{A_0} \times 100 \quad (4)$$

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Where A_0 was absorbance of blank (without extract) and A_s was absorbance with extract. The IC_{50} was calculated using linier regression of the samples and ascorbic acid was used as the positive control.

3. Results and discussion

3.1 Effect of solvent selection on yield

The percentage of extract obtained from the three solvents is shown in Figure 3. It can be seen that the use of ethanol gives the highest yield. The use of ethanol as solvent gives yield of 6.125% while water and 2-propanol give yield of 3.25% and 2.25%, respectively. Generally, different solvent will have different polarity. Polar solvent will extract polar metabolites while non polar solvent will tend to extract less polar metabolites. Polarity and viscosity of solvent will also influence the extraction of phytochemical compounds (Ballesteros *et al*., 2014; Thri *et al*., 2017). Solvent polarity index with similar phytochemical polarity index will result in high yield and extract quality (Wakeel *et al*., 2019). Ethanol and 2-propanol are organic solvents which have polarity index of 0.654 and 0.546, respectively. This index was less than water polarity index (10.2). Organic solvent are less polar than water is considered better to extract the phenolic compound (Ballesteros *et al*., 2014). The result of this research was similar with Ballesteros *et al.* (2014), that extract from organic solvent was higher than water. As an organic solvent, ethanol is also considered as less toxic solvent and relatively safer.

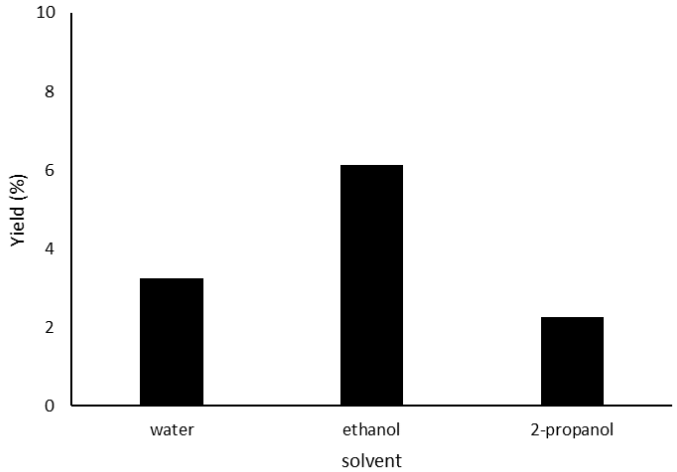

Figure 3. Yield of sappan wood extract using different solvents.

Result of HSP calculation of these three solvents are shown in Table 1. HSP was calculated for selecting/ predicting the suitable solvent to extract solute. Calculation of HSP for sappan wood used brazilin as solute, since brazilin is the major compound found in the sappan wood. If solvent and solute molecule has almost similar intermolecular forces, the solvent is usually good for extracting the solute. Prediction of HSP used the group contribution method proposed by Stefanis and Panayiotou (2008). The Ra number can be used to detect the similarities of solvent-solute intermolecular forces; hence it can be used to predict the suitability of solvent (Stefanis and Panayiotou, 2008). Smaller R_a means that solvent and solute has higher affinity. It was observed from Table 1 that the R_a of brazilin combination with the three solvents were different. Ethanol has the lowest R_a and it means that solute and solvent has closer distance or more similar intermolecular forces comparing with water and 2-propanol. The use of ethanol then will give better interaction between solute and solvent; thus, it will improve the amount of extract gained.

Table 1. HSP calculation between brazilin and solvent.

Solvent/solute	δ_D	δ_P	δ_H	R_a
Water	15.5	16	42.3	24.9
Ethanol	15.8	9.1	20.3	1.9
2-propanol	15.8	6.1	5.3	3.3
Brazilin	15.6	8.5	18.6	-

The use of UAE as new method in extraction process also plays significant role. The UAE give cavitation, thermal, mechanical and physicochemical effects to the solute (Mahindrakar and Rathod, 2022). Cavitation happens in UAE is called acoustic cavitation (Sharma and Dash, 2022) which consist of symmetric and asymmetric cavitation. Symmetric cavitation will accelerate micro turbulence that increase the mass transfer, while asymmetric cavitation creates vapor bubbles to break the biomass (Mahindrakar and Rathod, 2022). This whole effect will make the cell wall of solute destroys easier and the release of active compounds will be more effective (Yang *et al*., 2021). Most of the conventional method need several hrs to get the extract, but using UAE, the extraction time can be reduced only in several minutes to 1 hr. Arsiningtyas (2021) used 24 hrs of maceration time using 50% ethanol to get the yield of 8.7%, while in this research, although the ethanol yield was still less (only 6.125%) but it just needs 15 minutes of extraction time. The time comparison was also in accordance with those by Milićević *et al.* (2021) who reported similar efficiency of 10 minutes UAE with 24 hrs of conventional extraction method.

3.2 Effect of solvent on color

The color of extract was shown in Figure 4. The ethanol and water extracts tend to have red color, while the 2-propanol extract has yellow-orange color. Brazilin is colorless compound but the oxidation of brazilin is very easy to happen and it will change the brazilin into brazilein. Compared with brazilin which is colorless, brazilein has natural color that bears from yellow to red. When comparing the extract gained with the color standard (Figure 2), it can be concluded that the use of water as solvent gives extract color which is close to

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pearl ruby red, while ethanol extract is between pearl ruby red-traffic red and color of 2-propanol extract tends to traffic red.

Figure 4. Color of the sappanwood using solvents: (1) distilled water, (2) ethanol and (3) 2-propanol.

The differences of color are influenced by the pH of solution (Rahayuningsih *et al*., 2018). Acid condition will shift the color into dahlia yellow, while the base condition will change the color into ruby red. The change of color due to acid-base condition is common for polyphenolic pigment. Acid and base condition will cause protonation and deprotonation. When the solution is base, hydroxyl ion of the base will cause deprotonation of hydroxyl group in brazilein and it resulted in variation of electron localization that makes the different color (Ngamwonglumlert *et al*., 2020). The low pH will cause the color of yellow, while increasing pH will change the color into orange and red. Increasing pH also will increase the darkness of the color. pH of the solutions was 6.6, 5.2 and 4.9 for water, ethanol and 2-propanol, respectively. Water has the highest pH among other two solvents, therefore it has the red color and tends to be darker than others. Ethanol and 2-propanol have lower pH, then the color will change into traffic red and dahlia yellow. Rahayuningsih *et al.* (2018) reported that although the visual color looks different, but actually the absorbance of the color is almost same. The change only happened in the color intensity of the extract where acid condition will decrease the color intensity. Low pH was mentioned to have more stable color of brazilein and higher pH will reduce the color stability (Ngamwonglumlert *et al*., 2017; Ngamwonglumlert *et al*., 2020).

3.3 Effect of solvent on phytochemical compound

The qualitative phytochemical compound analysis of the extract was tabulated in Table 2. From the result, the differences of solvent didn't give effect on the phytochemical compound of the extract. All of the three extracts contained saponin, tannin, flavonoid and triterpenoid, while alkaloid and steroid were not detected. It might be caused by the polarity of solvents used. Water, ethanol and 2-propanol belong to polar solvent and tend to extract the polar metabolites. This result was different with Arsiningtyas (2021) where the extract contain alkaloid and steroid but triterpenoid and tannin were not detected. But this result was similar with Srinivasan *et al.* (2012) where alkaloid was not detected in the extract. The differences of phytochemical compound compared with another research could be because of the differences of the growth place of the plant. Different growth place will cause differences in such conditions like soil, nutrient, sun radiation and even potential bacterial attack. Those conditions will affect the production of phenolic compounds since phenolic compounds are usually produced as defense response of the plants to survive (Arsiningtyas, 2021; Herrera-pool *et al*., 2021).

Table 2. Qualitative analysis of phytochemical compound.

Solvent	Alkaloid	Saponin	Tanin	Flavonoid	Steroid	Triterpenoid
Distilled Water	-	+	+	+	-	+
Ethanol	-	+	+	+	-	+
2-propanol	-	+	+	+	-	+

3.4 Effect of solvent on total phenolic content and antioxidant activity

Phenolic compounds can be found in many plants. These components usually are reactive as hydrogen donor and therefore they can act as good antioxidant (Vankar and Srivastava, 2010). Antioxidant plays an important role for human being since its capacity to prevent many diseases that are related with oxidation such as cancer, diabetes and cardiovascular disease (Thri *et al*., 2017). Hence phenolic compounds are considered as compounds which usually have antioxidant activity. The TPC and antioxidant activity of the extract of sappan wood was shown in Table 3. From Table 3 it can be concluded that there was correlation between TPC and its antioxidant activity. Higher TPC will result in higher antioxidant activity. In this research, TPC and antioxidant activity for the three solvents are: ethanol > 2 -propanol > water. Ethanol has the highest TPC and lowest IC_{50}. The same result were reported for the extraction of Limnopilla aromatic where ethanol gave the highest phenolic content, although another research found that combination ethanol-water gave highest phenolic content (Ngo *et al*., 2017). The change in solvent polarity was reported to affect the dissolution of phenolic compound and this will impact to its antioxidant activity (Thri *et al*., 2017). The antioxidant activity was reported in IC_{50} where it means antioxidant needed to decrease DPPH concentration by 50%. The lower IC_{50} of the compound means higher antioxidant activity. The IC_{50} of the extract also was compared with

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the IC_{50} of the ascorbic acid as standard. The IC_{50} of all three extracts were still higher than ascorbic acid that it means that their antioxidant activity was still lower than ascorbic acid. But all of three extracts has $IC_{50} < 50$ ppm, where $IC_{50} < 50$ ppm belongs to very active antioxidant (Arsiningtyas, 2021).

Table 3. TPC and antioxidant activity of extracts.

Solvent	TPC (mg GAE/100 g)	IC_{50} (ppm)
Distilled Water	221.69±0.06	5.79
Ethanol	254.14±0.05	3.19
2-propanol	228.59±0.06	4.08
Ascorbic acid (control)	-	2.61

4. Conclusion

From the result obtained, it can be concluded that ethanol is the best solvent for extracting phytochemical component of sappan wood extract using UAE. The HSP prediction of ethanol as solvent was in accordance with the yield resulted. Ethanol gave the yield of 6.125% with the color tends to between ruby red and traffic red. All of the three extracts contained saponin, tannin, flavonoid and triterpenoid. Ethanol also gives the highest TPC (254.14 mg GAE/100 g) and lowest IC_{50} (3.19 ppm).

Conflict of interest

The authors declare no conflict of interest.

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