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CHARACTERISTICS OF SAPPAN WOOD *(Caesalpinia sappan L.)* EXTRACTION USING THE HYDRODYNAMIC CAVITATION METHOD AND ITS POTENTIAL AS AN INDICATOR FOR AMMONIA ANALYSIS

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ABSTRACT. Sappan wood (Caesalpinia sappan L.) contains phenolic compounds, such as brazilin, that can be used as colour indicators. The bioactive ingredient of sappan wood will decrease its sensitivity as a colour indicator if it experiences oxidation due to heating, so selecting an appropriate extraction method is necessary. Extraction using hydrodynamic cavitation (HC) is considered environmentally friendly because it requires less energy, extraction process without heating, cheaper equipment, and can be easily scaled up for large-scale production. In a hydrodynamic system, cavitation is caused by a pressure drop through a narrow gap in a closed loop. This study investigated how extraction time, airflow rate, and ethanol concentration affect HC extraction results from Sappan wood. The HC extracts were characterized using total phenolic content (TPC) measurements, Fourier Transform Infrared (FTIR), UV-Visible (UV-Vis) spectrophotometer, and High performance liquid chromatography (HPLC). Results showed that an extraction time of 60 min, an airflow rate of 2L/min, and 60% ethanol as the solvent yielded the best TPC result of 8.22 ± 0.07%. The FT-IR spectrum of the HC extract showed several peaks, especially at 1,029 cm-1, 1,455 cm-1, 1,603 cm-1, 2,921 cm-1, and 3,320 cm-1, which characterized phenolic compounds. The UV-VIS spectrum of the HC extract shows two maximum absorption wavelengths (λ max), recorded at 450 and 540 nm in the initial form of the extract at pH 5.25. Brazilin content in sappan wood extract by HPLC was 70.63 ± 0.76 mg/g. HC extraction yielded a higher TPC content than the maceration method. Using the HC extract as a colour indicator in ammonia analysis produced good results, with an analysis recovery rate (%R) ranging from 101 to 105%.

INTRODUCTION

The choice of extraction technique is crucial because it affects the reliability and quality of subsequent analytical activities. The main goals of extraction are to be economically viable, environmentally friendly, and quick, and to produce high yields of bioactive compounds without compromising their biological activity. Several promising modern green extraction methods, such as supercritical fluid extraction, ultrasonic extraction, accelerated solvent extraction, and enzyme-assisted extraction, have gained increasing attention (Bitwell *et al.*, 2023). Supercritical CO_2 extraction is advantageous because it is a cleaner, more environmentally friendly method, however, this method has drawbacks including higher costs, complex equipment, the need for high pressure, and suboptimal performance when extracting polar compounds (Armani and Susanti, 2022). The ultrasonic extraction method utilizes high-intensity ultrasonic waves to produce acoustic cavitation in the liquid, thereby increasing the extraordinary mass transfer of the target compound into the solvent (Djaeni *et al.*, 2021). Another extraction method using the principle of cavitation is the hydrodynamic cavitation method. Compared to ultrasonication, the hydrodynamic cavitation method requires less energy, is less expensive, and is easy to develop on a large scale (Setyawan *et al.*, 2020).

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Hydrodynamic cavitation is based on the interaction between fluid velocity and pressure. Bubbles arise when pressure drops below the liquid's vapor pressure at a constriction. Cavitation events, such as bubble formation, expansion, and rapid bursting, result from increased flow velocity produced by decreased flow pressure in a narrow gap in a closed flow system (Verdini *et al*., 2021). Extraction by hydrodynamic cavitation (HC) occurs through the interaction of materials with cavitation bubbles that produce hot spots and shock waves that can damage cell membranes and increase mass transfer, facilitate water access to damaged cells, and extract water-soluble compounds into the aqueous phase (Ciriminna *et al*., 2023). This approach is effective for solid matrix types, such as wood (Verdini *et al*., 2021), microalgae (Setyawan *et al*., 2020), and basil leaves (Wu *et al*., 2019). This study aimed to investigate the performance of the HC method using a nano bubble generator to extract bioactive compounds from sappan wood. Studies on the extraction of active compounds from sappan wood using the hydrodynamic cavitation method have not been published; therefore, additional research is needed.

The natural dye sappan wood (*Caesalpinia sappan L.*) is abundant and readily available in the environment. It contains brazilin, a polyphenol compound with potential as a natural dye. The hydroxyl group of the brazilin is subject to rapid oxidation. The transformation into a carbonyl group instigates structural alterations, resulting in the formation of brazilein, a molecule distinguished by its distinctive color. Brazilin oxidation is caused by changes in pH and temperature (Ngamwonglumlert *et al*., 2020). Brazilin, which can experience color changes in different pH environments, makes sappan wood extract potentially applicable as a color indicator. The bioactive ingredient of sappan wood will decrease its sensitivity as a color indicator if it experiences oxidation due to heating; therefore, selecting an appropriate extraction method is necessary.

This study aimed to examine the performance of a dynamic cavitation method using a nanobubble generator with a loop flow nozzle (Venturi tube) on sappan wood samples. The device works by flowing fluid in a straight line without causing any change in the flow direction, resulting in a mixture of nano and microbubbles. Variables that affect the extraction results include the selection of solvents, extraction time, airflow rate, and extraction methods. The sappan wood HC extracts were characterized using total phenolic content (TPC) measurements, Fourier-transform infrared (FTIR) spectroscopy, UV-Visible (UV-Vis) spectrophotometer, and high-performance liquid chromatography (HPLC). The potential of sappan wood extract as a color indicator in ammonia analysis in wastewater samples will then be studied using the titration method according to American Public Health Association (2023). The main basis for selecting a color indicator in titration is the suitability between the pH trajectory of the indicator color change and the pH at the reaction equivalence point. The performance of sappan wood extract as a color indicator in the titration method for ammonia analysis was compared with that of the indicator used in the standard method.

MATERIAL AND METHODS

Tools and materials

The sappan wood was obtained from a local market in Bogor. The solvents for extraction were distilled water and Emsure ethanol (Merck). The chemicals used for analysis were sodium carbonate (Merck), ammonium chloride standard (Merck) 1000 mg/L, and *Folin-Ciocalteu* phenol reagent (Merck) of pro-analytical quality.

Sappan wood extraction research was conducted using a series of tools, including a nanobubble generator developed based on the hydrodynamic cavitation method using a Loop Flow Nozzle (LFN). The resulting bubble sizes ranged from nanobubbles to microbubbles of several hundred micrometers. Other equipment used was a Branson 511 batch sonicator, Shimadzu UV-1280 spectrophotometer, HACH 3900 spectrophotometer, Selecta ProNitro II nitrogen distillation apparatus, DHG 9053A oven, thermoscientific 200-1000 µL micropipette, Sartorius BSA 224S analytical balance, Scilogex MS-H550-Pro stirrer heater, fourier transform infra red (FTIR) spectrophotometer Bruker Tensor 37, IKA RV 3 evaporator, and HPLC Shimadzu LC 20 AD.

Sappan Wood Extraction

The sappan wood was reduced in size using a dry blender and homogenized using a 60 mesh sieve. The samples were then stored in tightly closed containers and refrigerated for use in the next phase of the study. Two grams of sappan wood were added to two liters of distilled water. The sample mixture was inserted into the sample holder on the generator and flowed through a Venturi with a pressure booster (pump). Extraction treatments included variations in extraction time (10, 20, 30, 40, 50, and 60 min) and air flow rate (0, 1, 2, and 3 L/min). The best results were obtained for extraction using water, 60% ethanol, and 96% ethanol. The results of HC extraction were compared with those of maceration methods with stirring at 400 rpm and batch ultrasonics, with the same solvent composition and reaction time. The extract was filtered with Whatman No. 42 filter paper and evaporated at 40°C. The extract was dried using a blower oven at 40 °C. The dried extract was stored in a refrigerator for the next stage.

Characterisation of Sappan Wood Extract

The total phenolic content (TPC) of the sappan wood extract was determined using the Folin–Ciocalteu reagent (Insuan *et al*., 2024). An extract (0.5 ml was combined with 2.5 mL of FC reagent that had been diluted 1:10 in distilled water. After a duration of 5 minutes, 2 mL of a 7.5% (w/v) sodium carbonate solution was incorporated, and the mixture was allowed to stand at room temperature for 15 minutes. The absorbance was measured at 760 nm. Calculate the standard curve from the corrected blank values of the gallic acid standard. Calculate the total phenolic content as gallic acid equivalents using the regression equation between the gallic acid standard. Total phenolic content (TPC) was determined as gallic acid equivalents (GAE) per 100 grams of material (%).

Characterization of sappan wood extract includes measuring the TPC, identifying its functional groups using a Fourier Transform Infrared (FTIR) spectrophotometer, measuring λ_{max} and its absorption intensity (absorbance) using a UV-Vis spectrophotometer, and measuring the brazilin content using HPLC.

Testing the Potential of Sappan Wood Extract as a Colour Indicator for Ammonia Test

Sappan wood extract was studied as a color indicator for analyzing ammonia in water using the modified APHA 4500-NH_3C titration method (2023). This method uses 50 mL of a standard ammonia solution with a concentration of 25 mg/L. The sample was added to 6 N sodium hydroxide (NaOH) until the pH reached a minimum of 9.5, and then placed in an ammonia distillation apparatus. Next, 25 mL of distilled water was prepared, and 5 mL of 0.1% sappan wood extract was added. Distillation was carried out for seven minutes. The color spectrum of the distillate was examined by spectroscopy, and its pH value was measured. The distillate was then titrated with 0.02 N sulfuric acid. The ammonia value was then compared using a standard indicator, a mixture of methylene blue and methyl red in boric acid solution. The ammonia content was calculated using the following equation:

$$\text{mg } NH_3\text{-N/L} = \frac{(\text{ml sample} - \text{ml blank}) \times 280 \times N\ H_2SO_4}{\text{ml sample}}$$

Data analysis

Data analysis used a completely randomized experimental design with one-way Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS). If the ANOVA results showed a significant effect, a Duncan test was performed at a significance level of $\alpha = 5\%$.

RESULTS AND DISCUSSIONS

Sappan Wood Extraction

Sappan wood contains a variety of phenolic compounds, including brazilin, xanthones, flavones, and coumarins (Kurniasari *et al.,* 2023). A variety of factors influence the extraction of phenolic compounds from plant material. The extraction process factors that contribute to the extraction results include the extraction method, particle size, extraction duration, temperature, and solvent (Nirmal *et al*., 2015). The mechanical method is employed to enhance molecular interactions between the phenolic compound and the solvent.

Extraction by HC occurs due to the interaction of materials with cavitation bubbles that produce hot spots and shock waves, which can damage cell membranes, enhance mass transfer, allow water to penetrate damaged cells, and draw water-soluble compounds into the aqueous phase (Ciriminna *et al*., 2023). HC is based on the relationship between fluid speed and pressure. The phenomenon of cavitation, involving the formation, growth, and explosion of bubbles, happens suddenly because the flow speed increases due to a pressure drop in a narrow gap (nozzle) within a closed system. The formation of bubbles can be affected by factors that narrow the cross-sectional area of the nozzle (Verdini *et al*., 2021).

This study will investigate hydrodynamic cavitation extraction of sappan wood using a loop flow nozzle (LFN) nano-bubble generator. The LFN operates with a straight fluid flow, creating a mixture of nano and microbubbles without altering the flow direction. The effects of extraction time, airflow rate, and solvent on the TPC of sappan wood during HC extraction were investigated.

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The Effect of Extraction Time on the Total Phenolic Content (TPC) of Sappan wood Extract

In this study, HC extraction was conducted in a homogeneous environment. The extraction solvent used was distilled water, and the solvent-to-sample ratio was kept constant at 2:2000 (g/mL). The extraction was carried out without the addition of air flow. The extraction results were expressed as the TPC. Figure 1 shows the data and graph of the extraction results.

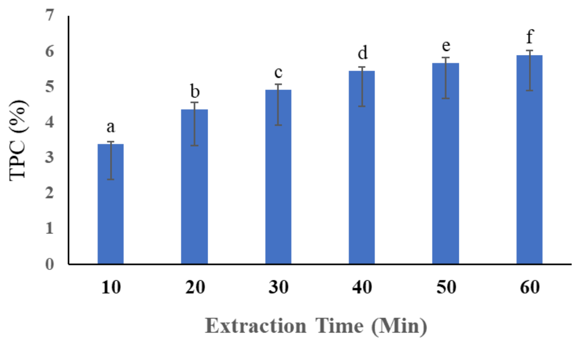

Figure 1. TPC content of HC extraction of sappan wood at various time variations

The water content of the sappan wood sample was 5.41%±0.08%. Significantly different results were obtained for each extraction time, which had a significant effect on the TPC content of the sappan wood extract. Hydrodynamic cavitation extract of sappan wood at extraction times of 10, 20, 30, 40, 50, and 60 minutes yielded TPC levels of 3.39±0.07%, 4.35±0.20%, 4.91±0.17%, 5.44±0.12%, 5.66±0.16%, and 5.89±0.12%, respectively. The best results were obtained after 60 minutes of extraction, yielding a TPC content of 5.89±0.12%. The sappan wood extract is bound within the wood tissue; therefore, it requires more time and energy to break down and diffuse into the solvent through the pores. The longer the extraction time, the greater the increase in the TPC value. However, it was observed that the longer the extraction time, the higher the solution temperature. This can result in increased oxidation in the extract (Septiani *et al.*, 2021). High heat and extended extraction durations can reduce brazilin and other soluble phytochemical substances (Insuan *et al.*, 2024).

The Effect of Air Flow Rate on the Total Phenolic Content of Sappan wood Extract

The sappan wood extraction process was studied with the addition of air flow rates (Qg) of 0, 1, 2, and 3 L/min, extraction time of 60 minutes, water and a sample ratio of 2 g/2000 mL. The system uses a fixed liquid flow rate (Ql) of 45–52 L/min. To create fine cavitation bubbles, the Ql/Qg value is usually designed at a minimum of 20, a higher Ql/Qg value will have an impact on the smaller bubble diameter and can produce a greater operating pressure (Levitsky *et al*., 2016). The Ql/Qg ratio affects the bubble size, and increasing the operating pressure also increases the bubble concentration (Alam *et al*., 2022).

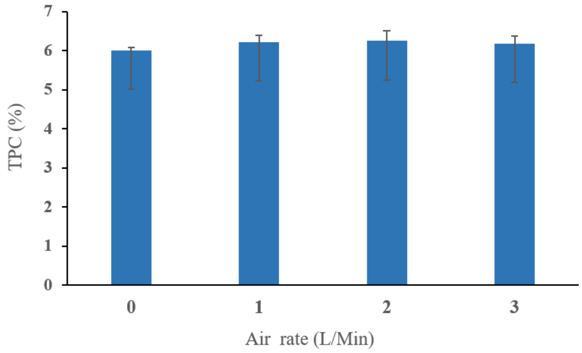

Figure 2. TPC content of HC extraction of sappan wood at various air flow rates

The Influence of Solvents on the Total Phenolic Content of Sappan Wood Extract

The effect of the solvent on HC extraction results is shown in Figure 3. The HC extraction was performed using 60% ethanol, 96% ethanol, and water at an air flow rate of 2 L/min and an extraction time of 60 min. The 60% ethanol solvent concentration yielded significantly different and higher TPC results, namely 8.22±0.07%, compared to water solvent (6.25±0.04%) and 96% ethanol (7.59±0.05%).

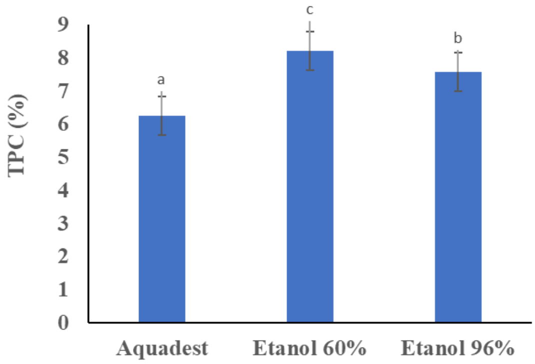

Figure 3. TPC content of HC extraction of sappan wood in various solvent variations

The phenolic part of the compound is polar. The phenolic content of sappan wood is found in the interior of the wood; therefore, the solvent must be able to penetrate the walls and membranes. Pure ethanol penetrates the walls and membranes more slowly than a water-ethanol mixture (Kurniasari *et al.*, 2023). The 60% ethanol solvent yielded better than 96% ethanol or water (Yuniati *et al.,* 2023).

Comparison of Hydrodynamic Cavitation Method with Maceration and Batch Ultrasonic Methods

The study compared the effectiveness of hydrodynamic cavitation extraction against maceration and batch ultrasonication techniques. The extraction method can affect the selectivity of the phenolic compounds.

The Ultrasonic Extraction (UAE)

This method offers several advantages, such as enhanced extraction efficiency, increased productivity, decreased solvent usage, lower energy consumption, and reduced processing time (Insuan *et al.*, 2024). The HC method provides benefits in terms of lower energy, cheaper equipment investment, and ease of large-scale development (Setyawan *et al.*, 2020).

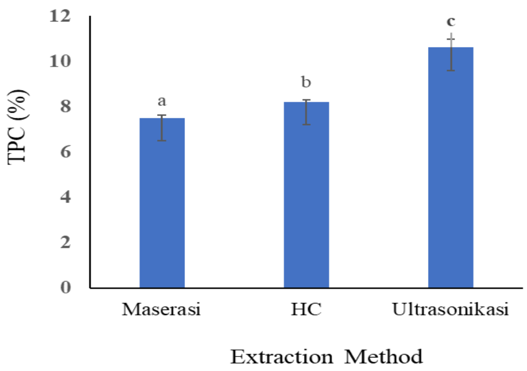

Figure 4. TPC content of HC extraction of sappan wood using various method variations

Figure 4 shows that the performance of hydrodynamic cavitation is superior to that of the maceration method but still cannot exceed that of the ultrasonic cavitation method. The TPC results for each method are 10.61±0.06% (ultrasonication), 8.22±0.07% (HC), and 7.51±0.09% (maceration). Extraction of sappan wood using the ultrasonication method has been widely carried out with satisfactory TPC values, as reported by Insuan *et al.* (2024) with a TPC value of 14.46%, and Kurniasari *et al.* (2023) with a TPC value of 0.25%. Bubble formation in the HC equipment used was dominated by micro and macro bubbles, which may affect the performance of the extraction process. Larger bubbles can easily break and produce a weak micro-jet (Levitsky *et al.*, 2016).

Characterisation of Sappan Wood Extract

The HC extract of sappan wood was analyzed for characterization using FT-IR spectroscopy to identify its functional groups. Figure 5 displays the FT-IR spectra showing several peaks, especially at 1,029 cm^{-1}, 1,455 cm^{-1}, 1,603 cm^{-1}, 2,921 cm^{-1} and 3,320 cm^{-1}. The peak around 3,320 cm^{-1} shows the characteristic peak of –OH stretching and 2,900–2,800 cm^{-1} is CH_3, CH_2 stretching, while the peaks at 1,603 cm^{-1}, 1,455 cm^{-1}, and 1,029 cm^{-1} are associated with C=C stretching of the brazilin skeleton, C–H bending, and C–O stretching vibrations, respectively (Nansu *et al.* 2023). The peak at 1,029 cm^{-1} was identified as a ring stretch of the phenolic compound. In the region between 1,455 cm^{-1} and 1,603 cm^{-1}, a C=C vibration peak was obtained (Hasrudin *et al.*, 2020). A weak peak at 1,697 cm^{-1} indicated the presence of a carbonyl group (C=O). This carbonyl group indicates the presence of some brazilein composition in the extract (Ngamwonglumlert *et al.*, 2020).

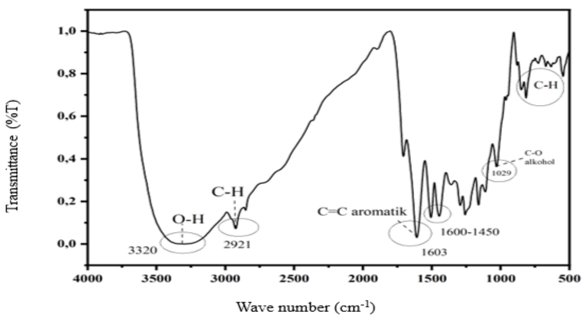

Figure 5. Results of FTIR spectrophotometer observations of sappan wood extract

The HPLC results revealed that the brazilin content in the sappan wood extract obtained using HC was 70.63±0.76 mg/g. Previous research has shown that the sappan wood extracted with one hour ultrasonic-assisted extraction (UAE) yielded the lowest amounts at 130.31 ± 2.74 mg/g, sappan wood obtained through reflux (15 hours) and maceration (3 days) produced the most notable amounts of brazilin, measuring 185.30 and 183.76 mg/g of dry extract, respectively (Insuan *et al.*, 2024). The HC extraction using HC showed a relatively high brazilin content achieved through a short extraction process.

The pH and color of the sappan wood extract were analyzed using a UV-Vis spectrophotometer, with absorption measured at wavelengths between 320 and 800 nm. The color observations are connected to the structural characteristics of the brazilein molecule, as indicated by the UV-VIS spectrum. In the initial form

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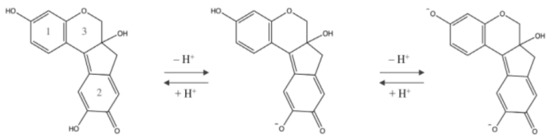
of the extract at pH 5.25, two peak absorption wavelengths (λmax) were recorded at 450 and 540 nm, as illustrated in Figure 7. At the lowest pH value, there may still be some presence of brazilin, which shows a λmax of 450 nm, and this brazilein form shows a yellow color. The solution at pH 6.47, which is orange, exhibits a peak at 530 nm, but the λmax 450 nm peak is not visible (form 2). The solution at pH 8.25 with a red color consisted of form 3 brazilin with λmax 520 nm with more intensity, and a peak began at 325 nm. The change in solution color at various pH levels results from modifications in the molecular structure triggered by the deprotonation process (Figure 6). At increasing pH values, the structure of brazilein moves toward a completely deprotonated form. Conversely, its structure returns to a form with a zero neutral charge at lower pH values (Ngamwonglumlert *et al*., 2020).



Form 1 Form 2 Form 3

Figure 6. Possible forms of brazilein at different pH (Rondão *et al.,* 2013)

Potential Application of Sappan wood Extract as a Colour Indicator in Wastewater Ammonia Analysis

Sappan wood extract was applied as a colour indicator in the ammonia analysis process using the titrimetric method by APHA (2023). The principle of this method is to measure the ammonia gas formed during the distillation and alkalinization of an ammonia sample. The alkaline nature of ammonia gas changes the pH of the indicator in the distillate. This change in pH changes the color of the indicator. Higher ammonia levels increase the pH and color intensity.

Sappan wood extract with a concentration of 0.1% (w/v) was added to distilled water as a container for ammonia from the distillation process during the analysis. The performance of the sappan wood indicator was compared with that of the standard method, which is a mixture of methylene blue and methyl red indicators. The analysis results are shown in Table 1. The standard method uses boric acid as a container for the distillate. Boric acid cannot be used in sappan wood extract because it is more stable at low pH levels, which reduces its sensitivity as an indicator. However, this has a positive impact because it reduces the need for hazardous chemicals.

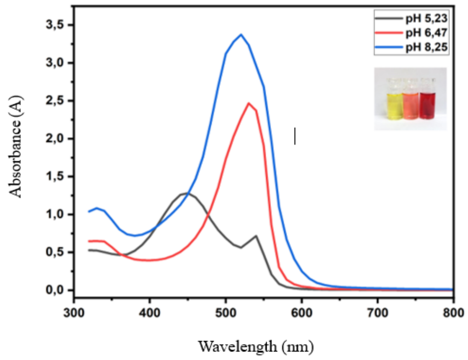


Figure 7. UV-VIS spectrum of brazilein solution at different pH

Table 1 presents data on applying sappan wood extract as a color indicator in ammonia analysis. The analytical recovery of sappan wood extract was 101–105%, whereas that of the standard indicator was 99–100%. The acceptable limit for standard recovery according to APHA (2023) is 90–110%, indicating that the sappan wood extract performs well as a color indicator for ammonia analysis.

Table 1. Performance test data of sappan wood extract as a colour indicator in ammonia analysis

Ammonia Level (mg/L)	Sappan wood Indicator		Standard Indicator	
	Ammonia Level (mg/L)	% Recovery	Ammonia Level (mg/L)	% Recovery
15	15.68	105	14.92	99
50	50.68	101	49.73	99
100	105.19	105	100.41	100

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

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Extraction using the HC method provides an alternative method for extracting sappan wood. The extraction of natural dyes from sappan wood through the HC method yielded optimal results under conditions of a 60-minute extraction duration, an air flow rate of 2 L/min, and a 60% ethanol concentration yielded the best TPC result of 8.22 ± 0.07%. The performance of the HC method was better than that of the maceration method, but not yet superior to that of the ultrasonication (UAE) method with the same extraction time.

Characterization of sappan wood extract showed color changes with increasing pH. The observed changes were yellow at acidic pH (5.25), orange at neutral pH (6.47), and reddish at alkaline pH (8.25). The FT-IR spectrum of the HC extract showed several peaks, especially at 1,029 cm^{-1}, 1,455 cm^{-1}, 1,603 cm^{-1}, 2,921 cm^{-1}, and 3,320 cm^{-1} which are characteristic of phenolic compounds. The UV-VIS spectrum of the HC extract shows two maximum absorption wavelengths (λ max), recorded at 450 and 540 nm in the initial form of the extract at pH 5,25. Brazilin content in sappan wood extract by HPLC was 70.63 ± 0.76 mg/g. These characteristics were used to test the performance of sappan wood extract as a color indicator in ammonia analysis. The sappan wood extract performed well as a color indicator for ammonia analysis, with a % recovery analysis in the 101─105% range. The acceptable limit for standard recovery according to APHA (2023) is 90–110%, indicating that the sappan wood extract performs well as a color indicator for ammonia analysis.

Recommendations

The performance of hydrodynamic cavitation extraction in sappan wood using the loop flow nozzle (LFN) method requires further improvement. The rotating flow nozzle type can accelerate the microbubble formation process; therefore, further research is needed on sappan wood extraction using the rotating flow nozzle hydrodynamic cavitation technique in order to improve extraction yields.

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