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Extraction Of Brazilin From Sappan Wood as a Natural Reagent for Borax Detection by Digital Imaging Analysis

Zuri Rismiarti [1]✉, Mamat Rahmat[2], Asep Iin Nur Indra[3], Amanda Afifah Syaindi[4]
[1-4],Poltekkes Kemenkes Bandung, Indonesia
✉ zuri.tlm@staff.poltekkesbandung.ac.id, Phone: +6281907534643

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Abstract

The use of brazilien for borax detection is because the chemical properties of this compound are greatly influenced by pH which results in a change in the color of the brazilien. This compound gives an orange color at a pH range of 6-7 and yellow at an acidic pH, while at a pH range of 7-10 it gives a red color and at a pH >10 it gives a purplish red color. This research uses Brazilian extract from sappan wood for borax detection. By utilizing this compound extract, the use of chemical reagents for the detection of borax in food samples can be minimized. Apart from that, it can increase the added value of these crop commodities and can take advantage of the abundant natural resources in Indonesia. This research aims to develop a method that is accurate, simple and cheap and does not use special instruments to diagnose borax based on digital imaging, which includes extraction of brazilien from sappan wood using a maceration method using 96% ethanol solvent and water with a UV-Vis spectrophotometer, ratio optimization brazilin extract with boric acid, optimization of measurement time. The results of the research show that the solvent that provides optimum absorbance for brazilin extraction is water or distilled water, with a ratio of brazilin extract and boric acid of 1:1 and the measurement time is 5 minutes.

Keywords: Brazilin; Borax; Imaging; Digital

INTRODUCTION

Borax is a compound with the chemical name sodium tetraborate or borax salt ($Na_2B_4O_7.10H_2O$) and boric acid (H_3BO_3). Borax is widely used by small industries or home industries, in the manufacture of noodles, gendar, or gendar crackers (rice crackers), lontong (as a hardener), ketupat (as a hardener), meatballs (as a thickener and preservative), soy sauce (as a preservative), and even making chicken porridge (as a thickener and preservative) (Kresnadipayana, 2017) with borax levels between 0.08%-0.29%. raders use borax in products meatballs with the highest content of 2.32mg/g sample (Misbah, 208). Consumption of foods containing borax will cause brain, liver, fat and kidney disorders, fainting and even death. Therefore, the Regulation of the Minister of Health of the Republic of Indonesia prohibits the use of borax in food. But in reality, borax is often added to food according to data from the Food Safety Survey of the Indonesian Food and Drug Administration in 2010, which stated that borax abuse amounted to 8.80%. These foods are usually produced by small and medium enterprises (SMEs) that are not registered with the Food and Drug Administration (POM) so that the quality is less controlled. Therefore, routine monitoring of borax levels in food is needed so as not to endanger consumers so that food circulating in the community maintains its quality and is safe for consumption.

An effective technique to determine borax levels in food in the field is the use of test kits. The test kit that has been developed uses brazilien extract from the sappan wood plant (Caesalpinia sappan Linn). The use of brazilien for borax detection is due to the chemical nature of this compound is strongly influenced by pH which results in a change in the color of brazilien. This compound gives orange color at pH range 6-7 and yellow color at acidic pH while at pH range 7-10 gives red color and at pH >10 gives purplish red color. This study used brazilien extract from sappan wood for borax detection in food. Research conducted by Wahyuni (2020) developed borax analysis using brazilien extract from sappan wood (Caesalpinia sappan Linn) using a UV-Vis spectrophotometer. The results of the brazilien extraction were able to detect borax levels in food based on a purplish red color complex in the range of 0.031-0.25 ppm borax. Borax in the pH range of 7-10 boron ion species from Na_2B_4O7 which is most dominant is sodium tetraborate $B_4O_5(OH)_4^{2-}$. The brazilien compound gives an orange color in the pH range 6-7 and is yellow at acidic pH while in the pH range 7-10 gives a red color and at pH> 10 gives a purplish red color (Ulma et al, 2018). The extraction of brazilien compounds from sappan wood can be done by maceration method with a ratio of 1: 50 (g/mL) with 90% ethanol solvent (Putri et al, 2018).

The PAD method has been widely developed as an attractive tool for field analysis with good sensitivity, offering low cost, easy and portable applications encouraging its use in various diagnostic, health, and environmental fields. The PAD technique has been developed for metal diagnostics of Whatmann paper as a sensitive and selective device based on colorimeter sensors dripped by samples and reagents. This method has potential as a Point of Care (POC) diagnostic applied in healthcare (Chaiyo, 2015; Busa, 2016).

In this study, the extraction of brazilien extraction from sappan wood by maceration method using 90% ethanol solvent. The analysis device used with PAD technique uses Whatmann No. 42 paper that has been prepared to provide hydrophobic boundaries with cranyon wax. Colorimetric-based analysis method by taking photos through an android-based cellphone. The photo results were measured for color intensity using the Image J program. The intensity data was converted to absorbance using the Lambert-Beer equation. This research is focused on making a practical, simple and inexpensive method that does not use special instrumentation to diagnose borax using digital imaging-based PAD. The parameters studied include characterization of brazilien extraction results using UV-Vis spectrophotometer and digital imaging, optimization of measurement time, and optimization of brazilin extraction ratio with borax.

METHOD

Materials and Tools

The materials used in this study include sappan wood, 96% ethanol (E-Merck), (E-Merck), NaOH (E-Merck), $Na_2B_4O_7$. $10H_2O$, (E-Merck), distilled water. The chemicals used are pro-analysis.

The equipment used in this analysis are Whatman Paper No.42, cranyon wax, 10 mL measuring flask, 1 mL measuring pipette, 10 mL measuring pipette, 50 mL beaker, analytical balance, electric heater, UV-Vis spectrophotometry.

Research Procedure

Extraction of Brazilin from Sappanwood

Weigh 30 g of sappan wood simplia on an analytical balance, then blend the sappan wood simplisia until it becomes powder. Then the sappan wood powder was sieved using a sieve. Then maceration was carried out in a ratio of 1: 50 (g/ml) with 90% ethanol as a solvent. The maceration process of sappan wood powder yield was carried out for 48 hours at room temperature and protected from sunlight. The extraction results were characterized by brazilin with a UV-Vis spectrophotometer.

PAD-borax Making Procedure (Staining Pattern Making)

Paper-based devices were designed on Whatman No. 42 paper that had been patterned in a rectangular shape with a size of 5x2 cm. The hydrophobic boundary was made using cranyon wax on the circle side. Furthermore, the filter paper was heated on a hot plate at 120^{o}C for 5 minutes so that the wax could penetrate to form a hydrophobic boundary. The resulting paper was used as a paper device for the analysis of the next procedure. Data Interpretation for Digital Imaging

The blue-red color formed from brizalin-borate in each parameter was photographed with an android phone, then the results were processed using Image J software 1.48. Then the intensity value that appears is converted into absorbance value, using Lambert-Beer Law (equation 1). For each color on the paper, the RGB value is determined.

$$A = -\log\left(\frac{I}{I_0}\right)$$

Description: A is the absorbance; I is the intensity of the sample or control and Io is the intensity of the solvent with a value of 255. This result is used for the next treatment.

Optimization of Reagent Ratio of Sappan Wood:Boric Acid 1:1; 1:2; 1:3; 1:4

Optimization of Sappan Wood:Boric Acid Reagent Ratio of 1:1; 1:2; 1:3; 1:4. The next treatment is the same as the way the staining pattern is made. The sharp color intensity was used as the optimization of Sappan Wood:Boric Acid Reagent Ratio of 1:1; 1:2; 1:3; 1:4. This result was used for the next treatment.

Measurement time optimization

Measurement optimization was carried out by varying the measurement time including: 2, 5, 10, 20, 30, 90 minutes. The next treatment is the same as the pH optimization work with the results using the optimum results. Sharp color intensity is used as measurement time optimization. This result is used for the next treatment.

RESULT AND DISCUSSION

Extraction of Brazilin from Sappanwood Digital Imaging

Extraction of brazilin from sappan wood using maceration method is shown in Picture 1 using 96% alcohol with distilled water.

(a) **(b)**

Picture 1: (a) Extraction of Brazilin by Maceration Method; (b) Extraction Results with Water and 96% Alcohol Solvents

Based on Picture 1, the extraction using water solvent is more concentrated compared to 96% alcohol with the resulting color is yellowish red for water solvent and yellow for 96% alcohol. This is also shown from the results of digital imaging and UV-Vis spectrophotometry presented in Table 1 and Table 2.

Table 1. Absorbance of Brazilin Using Water and 96% Alcohol Solvents by Digital Imaging Analysis

Solvent (5x)	Color Intensity			Absorbance		
	R	G	B	R	G	B
Aqua DM	138.142	57.621	10.629	0.26621	0.64595	1.380048
Alcohol 96%	123.483	64.193	5.915	0.31493	0.59905	1.634585

Table 2. Absorbance of Brazilin Using Water and 96% Alcohol Solvents with UV-VIS Spectrophotometric Analysis

Solvent (5x)	Wavelength (nm)					
	490	500	520	530	540	550
Aqua DM	0.74071	0.73635	0.7487	0.8847	0.9982	0.6819
Alcohol 96%	3.404	2.5082	0.777	0.5011	0.3666	0.2813

Based on Table 1 shows that brazilin extract with digital imaging analysis obtained the highest absorbance of blue color and in Table 2 shows UV-Vis spectrophotometric analysis of the highest absorbance at a wavelength of 540 nm. Both analyses show the highest absorbance by using distilled water solvent to extract brazilin from sappan wood. Sappan wood (Caesalpinia Sappan L.) produces a red pigment called brazilein. This pigment has a sharp and bright red color at neutral pH (pH 6-7) and shifts towards purplish red with increasing pH. The compounds included in this composite are brazilin, 3-O-methylbrazilin, and brazilein with brazilin as the main constituent of sappan wood extract (Robbani, 2022). Brazilin is very soluble in ethanol solvents and easily soluble in water solvents so that both can be used as solvents in the process of extracting brazilin from secang wood. Extraction using a water solvent is a conventional process and is easy to do. On an industrial scale, this method is stated to be more effective than special solvent extraction. This is because the process is simple and does not require high-tech equipment, so production costs can be reduced (.Failisnur F, 2019)

Optimization of Sappan Wood:Boric Acid Reagent Ratio 1:1; 1:2; 1:3; 1:4

The result of brazilin extraction from sappan wood using distilled water solvent was then used as a natural reagent for borax detection. This comparison uses the ratio of 1 drop of brazilin extract with 1 drop; 2 drops; 3 drops; and 4 drops of boric acid as the borax sample to be detected. The optimization result of the ratio of brazilin reagent with boric acid is shown in Picture 2 with the color change from yellowish red from sappan wood extract to yellow after the addition of boric acid sample. Based on Picture 2, the yellow color formed between brazilin and boric acid extracts fades with the addition of boric acid drops and it can be seen that the 1:1 ratio between brazilin and sappan wood extracts is more concentrated than other ratios. Brazilin compounds in sappang wood has a hydroxyl group as an active site and an electron pair conjugated as a chromophore group so that it has the potential to be an anion sensor compound (Sari, 2017).

Picture 2: Reagent Ratio of Sappan Wood:Boric Acid 1:1; 1:2; 1:3; 1:4 (drops)

The photos formed between brazilin and sappan wood were analyzed by digital imaging to determine the ratio of brazilin extract to sappan wood which is presented in Table 3. Based on Table 3 shows that the optimum ratio is 1:1, namely 1 drop of brazilin extract reagent with 1 drop of boric acid. This is shown from the absorbance value formed is the highest among other ratios which is 0.512.

Table 3. Results of Analysis of Sappan Wood:Boric Acid Reagent Ratio by Digital Imaging

Analyte Ratio: Sappan Wood Reagent	Color intensity			Absorbance		
	R	G	B	R	G	B
1 : 1	155.653	121.867	78.402	0.214383	0.320654	0.512213
2 : 1	179.306	147.482	105.305	0.152945	0.237801	0.384091
3 : 1	189.114	158.782	119.186	0.129816	0.205739	0.330315
4 : 1	182.87	159.267	126.202	0.144398	0.204414	0.305474

This is in accordance with research conducted by Wahyuni that the ratio of secang wood extract to borax is in the ratio 1:1 using spectrophotometry UV-Vis (Wahyuni, 2020). The brazillin content in secang wood is an indicator to analyze the borax content by giving yellowcolor change depending on the concentration of borax in the sample

Optimum Time Optimization

The results of brazilin extraction from sappan wood using distilled water solvent with a ratio of 1:1 were then used to determine the optimum time optimization between brazilin reagent and boric acid. The results of the optimum time optimization of brazilin extraction reaction with boric acid are presented in Picture 3.

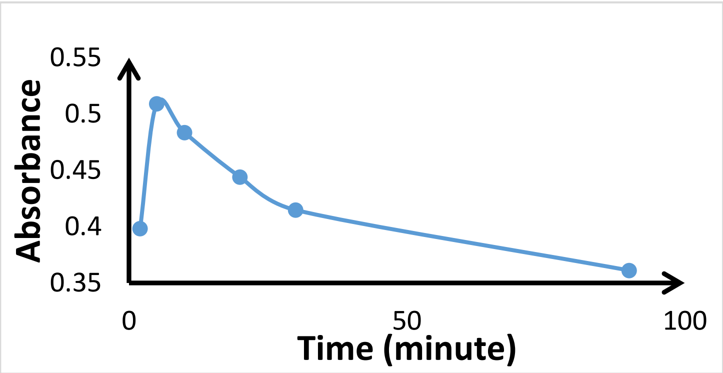
Picture 3: Optimum time optimization

The results of photos formed between brazilin and sappan wood analyzed by digital imaging to determine the optimum time are presented in Table 4. Based on Table 4 shows the optimum time of brazilin extraction with boric acid is 5 minutes. This is indicated from the absorbance value formed is the highest among other ratios of 0.509051. Based on research results, it shows that this method has a fast measurement time so that it can easily be applied in the field such as the use of curcumin paper in borax detection

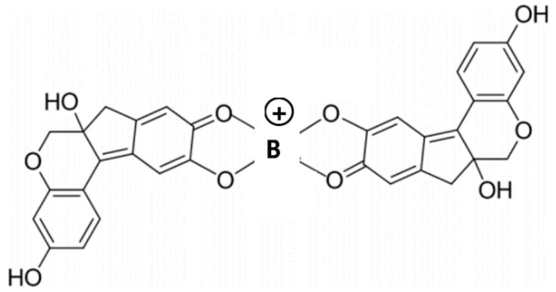
Table 4. Optimization of Optimum Time of Sappan Wood:Boric Acid by Digital Imaging

Time (Minute)	Color intensity			Absorbance		
	R	G	B	R	G	B
2	187.44	151.313	101.938	0.133678	0.226664	0.398204
5	160.291	125.553	78.975	0.201631	0.307713	0.509051
10	157.18	125.505	83.74	0.210143	0.307879	0.483607
20	161.396	132.548	91.725	0.198647	0.284167	0.444052
30	171.233	142.209	98.132	0.172953	0.253613	0.41473
90	176.852	149.41	111.02	0.15893	0.232161	0.361139

Based on Picture 5, the longer the observation time between brazilin extract and boric acid will have an impact on decreasing absorbance so that the optimum time between brazilin and boric acid with this digital imaging method is 5 minutes. This shows that the stability between brazilin and boric acid is influenced by time. The structure of borax complex with brazilin is shown in Picture 6.



Picture 5: Time Chart of Observation between Brazilin Extract and Boric Acid



Picture 6: borax complex with brazilin

CONCLUSION

The research used secang wood extract with a maceration method using water as a solvent. This secang wood extract has the potential as a reagent in borax detection. The measurement method at the fifth minute uses a ratio of secang wood extract and borax is 1:1. Further research can be carried out using sample applications and a validation stage can be carried out

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