

# CONTRIBUTION OF EXTRACTIVES TO THE BARK COLOR OF *Caesalpinia sappan*

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## ABSTRACT

The purpose of this study was to investigate the contribution of *Caesalpinia sappan* (sappan wood) bark extractives by analyzing color change of the bark after extraction and the color of the extracts with several color measurement methods. Successive extraction was performed with *n*-hexane, ethyl acetate, methanol, and hot water. Color change of the bark was measured using CIELab color system and the extracts were analyzed with Ultraviolet-Visible Spectrophotometer, total phenolic content (TPC), and Gas Chromatography Mass Spectrometry (GC-MS). The results showed that the highest change on the bark color after methanol extraction with the  $\Delta a^*$  and  $\Delta b^*$  values of  $-2,53 \pm 0,60$  and  $-3,64 \pm 1,20$  respectively. Also, methanol extract showed the highest total phenolic content ( $860,24 \pm 30,19$  mg GAE/g). In addition, the Ultraviolet-Visible is analysis showed a peak at 478 nm in the hot-water soluble extract and two peaks in the methanol soluble extract at 396 nm and 478 nm. Hydroquinone was detected as one of the major compounds by Gas Chromatography Mass Spectrometry in the methanol soluble extract. It was suggested that the color of *Caesalpinia sappan* (sappan wood) bark as well as the deep red coloration of its extract might be contributed by multiple phenolic compounds contained in the methanol extract with hydroquinone as its precursor. Therefore, it is also a potential source for coloring matter.

**Keywords:** *Caesalpinia sappan*, CIELab, color measurement, hydroquinone, pH, wood extractives, Ultraviolet-Visible.

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## INTRODUCTION

Sappan wood (*Caesalpinia sappan* L.), synonym sappan wood (*Biancaea sappan* L.), is a member of *Fabaceae* family distributed across some Asian countries such as Indonesia, China, India, Vietnam, Myanmar, Sri Lanka, and Malay Peninsula (Athinarayanana *et al.* 2016). It is a small-medium sized shrubby tree of about 10 m in height. Its dense branch and thorny reddish-brown bark have made sappan wood (*Caesalpinia sappan* L.) a good ornamental and hedgerow plant (Yulandani *et al.* 2015).

Sappan wood has been utilized as natural dyeing agent for foods, furniture, textile, and other decorative items in South East Asia and the products has been valued as a more eco friendly solution for dyeing (Nathan *et al.* 2021). Also known as Secang in Indonesia, sappan wood (*Caesalpinia sappan* L.) is used as natural dye and traditional herbal drink called Wedang Uwuh in the javanese community. The dyeing agent from this plant is extracted by boiling in water, which comes out as a red dye extract used on textile products including the traditional Batik textile (Widyasti *et al.* 2017, Vardhani 2019).

Holocellulose, which are the major components of bark and wood, do not give significant color on their own. However, secondary metabolites and their complexes with cell walls contribute more to the coloration of wood and bark (Chen *et al.* 2014). For example, naphthaquinone was speculated to be responsible for the coloration of *Diospyros* spp. heartwood (Yazaki 2015) and phenolic substances that undergo redox reaction which resulting in darker color in heated horsetail pine (*Pinus massoniana* Lamb.) wood (Wu *et al.* 2021).

In addition, previous studies on the bark color focused more on its ecological and physiological importance such as tree adaptation to temperature and photosynthesis capability of the stem (Harvey 1923, Pfanzen and Aschan 2001). Moreover, Widyasti *et al.* (2017) worked on the standardization of sappan wood (*Caesalpinia sappan* L.) bark extract as a natural dye through spectroscopy.

Phytochemical research of sappan wood (*Caesalpinia sappan* L.) heartwood extract has been done extensively. Phenolic acids, anthraquinone, and flavonoids were found to be the major constituents (Vij *et al.* 2023). Various bioactivities have been reported from sappan wood (*Caesalpinia sappan* L.) wood extracts such as antioxidant, antibacterial, anti-inflammatory, antiacne, hepatoprotective, and other medicinal properties such as anticancer, cytotoxic, anti-convulsant, and cerebral ischemia inhibition (Wan *et al.* 2019, Vij *et al.* 2023, Prashith *et al.* 2021).

Brazilin is one of the major compounds found in the wood of this plant and other species in *Fabaceae* family. It is usually colorless in nature but readily oxidized to a red colored dye known as brazilin (Dapson and Bain 2015). Wide variety of pharmaceutical and industrial utilization has made brazilin as one of the most important phytochemicals from *Caesalpinia*. This also make brazilin one of the export commodities of several South East Asia countries (Nirmal and Panichayupakaranant 2015). For a natural dye purpose, however, studies of brazilin and other colored compounds are rarely done in sappan wood (*Caesalpinia sappan* L.) bark.

Moreover, due to its thorn, the bark is often discarded as waste in utilization of sappan wood (*Caesalpinia sappan* L.) Based on the background, this research aimed at investigating the contribution of extractives to the color of sappan wood (*Caesalpinia sappan* L.) bark by successive extraction with *n*-hexane, ethyl acetate, methanol, and hot water, as well as determining the color expression of the bark extract with CIELab color space, Ultraviolet-Visible (UV-Vis), and major compound identification with GC-MS.

## MATERIAL AND METHODS

### Bark collection and extraction

Stem bark from a single sappan wood (*Caesalpinia sappan* L.) tree (9 cm breast height diameter) was obtained from Forest Research and Education of Wanagama I, Gunung Kidul District, Yogyakarta, Indonesia. Successive extractions by three organic solvents and hot water ranging from 0,0 polarity index (*n*-hexane), 4,4 polarity index (ethyl acetate), 5,1 polarity index (methanol), and 9,0 polarity index (water) to extract all non-polar to polar secondary metabolites (Abdel-Aal *et al.* 2015). Extractions were carried out by macerating about 100 g of the bark sample with 500 ml of solvents for 6 hours in a reflux apparatus. The solution was then evaporated to remove all solvent using IKA RV 10 Rotary Evaporator (IKA® Works (Asia), Malaysia) and the resultant extract was weighed (yield was calculated as percentage of oven-dried bark sample). The residue of

the bark sample from the first extraction (*n*-hexane) was subjected to the next extraction using fresh solvents (ethyl acetate, methanol, and hot water 100 °C) with the same procedure, successively. Lastly, 1 % sodium hydroxide (NaOH) was used to remove all the remaining secondary metabolites as well as low-molecular-weight carbohydrates (Longue *et al.* 2010, ASTM D1109-84 2001).

### Color measurements and pH values

Color measurements were conducted in both bark (CIELab) (Moya *et al.* 2012) and extracts (Spectrophotometer and Reichs-Ausschuß für Lieferbedingungen (RAL) standard color chart). The pH value of both bark and extracts were also determined due to the ability of pH that would affect the color and stability of some naturally occurring pigments (Wahyuningsih *et al.* 2016). The extract through *n*-hexane, ethyl acetate (EtOAc), MeOH, and hot-water was dissolved in 250 ppm with its respective solvent and then read between 300 nm - 700 nm by UV-V spectrometry (model SP-3000 Nano, Optima, Tokyo, Japan). The bark sample was also subjected to the CIELab system (Spectrophotometer NF333, Nippon Denshoku, Japan), characterized by three parameters;  $L^*$ ,  $a^*$ , and  $b^*$ . The  $L^*$  axis represents the lightness, which varies from 100 (white) to zero (black), while  $a^*$  and  $b^*$  are the chromaticity coordinates. In the diagram of CIELAB,  $+a^*$  is the red direction,  $-a^*$  is green,  $+b^*$  is yellow, and  $-b^*$  blue. Each sample was read before and after the extraction process. Then, the values of  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  were calculated from the difference in value before and after extraction. The total differences ( $\Delta E^*$ ) was calculated using the Equation 1.

$$\Delta E^* = \sqrt{\Delta L^* + \Delta a^* + \Delta b^*} \quad (1)$$

Both bark and its extracts were subjected to pH/ion tester pHTestr® 10 BNC (Oakton Instruments, USA). The pH value of the bark was measured by submerging 1 g of the bark sample, before and after extraction, into 20 ml of distilled water for 24 hours. The residue was filtered and the solution tested for pH.

### Total phenolic content

Phenolic compounds were reported to give high attribution to color in previous studies (Wu *et al.* 2021, Yazaki 2015). The total phenolic content (TPC) was measured through the Folin-Ciocalteu method according to Baba and Malik (2015). This involved a brief reaction of 0,5 ml of each extract in DMSO solution with 2,5 ml of 10 % Folin-Ciocalteu reagent. After 2 minutes, 2 ml of 7,5 % aqueous sodium carbonate was added and then incubated for 30 minutes under room temperature. The absorbance of each solution was measured at 765 nm with a UV-Vis spectrophotometer. The results of total phenolic content were measured in triplicates for each sample and expressed as gallic acid equivalent (mg GAE/g).

### GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used to detect and identify the precursor of major compound in the colored extract. The methanol extract sample was silylated according to Wijayanto *et al.* (2015). This involved dissolving 2 mg of the sample into 15 µl TMCS and 85 µl BSA. After 1 hour of incubation, the sample was evaporated and the dry extract was dissolved in 1 ml of MeOH. The Gas Chromatography-Mass Spectrometry (GC-MS) data were collected with a GCMS-QP 2010 (Shimadzu, Japan), which involved injecting 1 µl of silylated sample into the machine. The GC-MS condition include: Rtx-5MS capillary column (30 m x 0,25 mm I.D. and 0,25 µm; GL Sciences, Tokyo, Japan); column temperature from 70 °C (1 min) to 290 °C at 5 °C/min; injection temperature of 270 °C; detection temperature of 290 °C; acquisition mass range of 50-800 amu using helium as the carrier gas. The mass spectrum of sample was compared with the ones available in NIST library.

### Statistical analysis





The data obtained were subjected to One-way analysis of variance (ANOVA) and Tukey HSD post-test with SPSS version 25 (IBM USA) to determine the significance of solvent factor to the TPC value. Also, the correlations between the parameters were determined through linear regression.

## RESULTS AND DISCUSSION

### Extractive content, color measurements, and pH values

Organic solvents (*n*-hexane, methanol, and ethyl acetate) and water were successively used in this study to obtain extractives in all polarity ranges. In addition, 1 % NaOH was used to remove extractives which still bound tightly to the cell wall i.e. hemicellulose, sugars, and polymeric condensed tannins with high degree of polymeration (Longue *et al.* 2010, Sakai 2001). The extractive content by each solvent after successive extraction are shown in Table 1. Using organic solvents and water, the highest extractive yield was obtained with hot-water, at 7,39 %, while *n*-hexane soluble extract showed the lowest yield at 0,44 %. Then, the 1 % NaOH extraction produced an extractive yield of 13,92 %.

**Table 1:** Yield, color description, and pH value of sappan wood (*Caesalpinia sappan*) extracts with various solvents successively.

Solvent	Extractive yield (%)	Color <sup>1</sup>		pH value
<i>n</i> -hexane	0,44		(No color)	6,72 ± 0,04
EtOAc	1,72		Olive Yellow	5,46 ± 0,04
MeOH	6,80		Tomato Red	5,52 ± 0,02
Hot-water	7,39		Ochre Brown	6,24 ± 0,04
NaOH 1 %	13,92			

<sup>1</sup> Colors were described according to Reichs-Ausschuß für Lieferbedingungen (RAL) standard color chart

Visually, the high concentration of coloring matter was obtained in MeOH and hot-water extract, which described as Tomato Red and Ochre Brown color. The less polar compounds such as lipids, higher fatty acids, and terpenes are extracted with *n*-hexane and EtOAc, while the more polar ones such as phenolics, flavonoids, tannins, and sugars are extracted by polar solvents like MeOH and hot-water (Sakai 2001).

Results of this study showed that the coloring matter in the bark of sappan wood (*Caesalpinia sappan*) is dominated by polar fractions. In comparison with the extractive content of sappan wood (*Caesalpinia sappan*) heartwood from a previous work, the bark of sappan wood (*Caesalpinia sappan*) contains lower concentration of apolar fractions but higher concentration of polar fractions (Badami *et al.* 2003). Hence, the coloring matter from the wood of this plant is commonly associated with its polar extractives which suggests that the bark might be a better dye source in term of yield and color.

The color measurements and pH values of each extract are shown in Table 1. Dried *n*-hexane extract showed a fat-like yellow-brownish color, which turned clear after dilution. Also, dried EtOAc, MeOH, and hot-water extracts formed a very dark brown color. After dilution, EtOAc and hot-water extracts formed a brown colored solution, with a darker color in the hot-water extract. Moreover, a deep reddish-brown color was formed by MeOH extract.

The pH value of each fraction was lower than 7 or in a weak acidic range. The EtOAc extract showed the most acidic pH value at  $5,46 \pm 0,04$ , while *n*-hexane extract showed a pH value close to neutral at  $6,72 \pm 0,04$ .

Generally, extracts from all solvents were in the weak acidic range, where EtOAc showed the most acidic pH. This pH range was similar to that of casuarina (*Casuarina equisetifolia*) leaves (Silas *et al.* 2012). According to Wahyuningsih *et al.* (2016), the value of pH could influence the color and stability of some naturally occurring pigments. Previous research by Ulma *et al.* (2018) showed that sappan wood (*Caesalpinia sappan*) wood extract is also affected by pH. Value of pH could also affect the amount of polyphenol content where a previous research showed that as pH value increase, polyphenol content decrease, hence resulting in different color (Asfar and Asfar 2023).

In a weak acidic to neutral condition, sappan wood (*Caesalpinia sappan*) wood extract color shows traffic red to ruby red color which is close to the color shown by MeOH extract. However, the less colored pigments shown by *n*-hexane and EtOAc extracts is an indication that both fractions are dominated by colorless compounds.

### Color change in the bark after successive extraction

The color measurement with the use of CIELab system after each successive extraction are shown in Table 2 and illustrated in Figure 1. The brightness value ( $L^*$ ) of the bark decreased after *n*-hexane extraction, slightly increased after extraction with EtOAc, and then sharply decreased after hot-water extraction to  $-6,28 \pm 0,16$ .

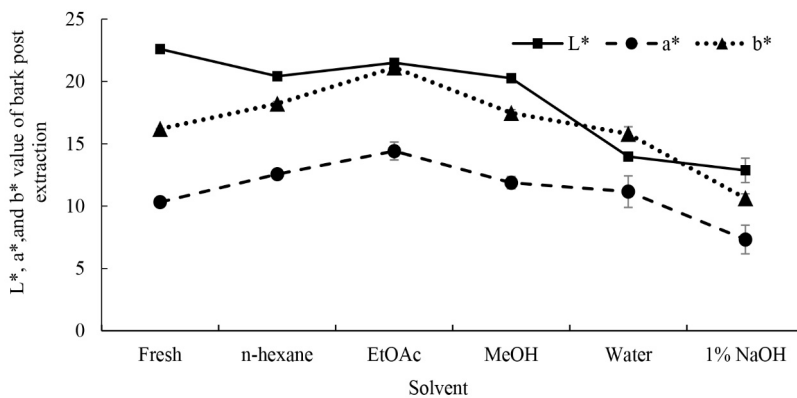
In addition, both redness ( $a^*$ ) and yellowness ( $b^*$ ) showed a similar trend, where it increased after *n*-hexane and EtOAc extraction, then decreased after extraction with MeOH and hot-water. Further extraction of the extractives with 1 % NaOH decreased all brightness, redness, and yellowness values. The highest color differences ( $\Delta E^*$ ) were observed after both hot-water and 1 % NaOH extraction at  $6,59 \pm 0,18$  and  $6,58 \pm 2,57$ , respectively. Also, the pH values of all bark samples were in a weak acidic range, as shown in Table 2.

**Table 2:** Color change and pH value of sappan wood (*Caesalpinia sappan*) bark after successive extraction.

Solvent used	Bark coloration change				pH value
	$\Delta L^*$	$\Delta a^*$	$\Delta b^*$	$\Delta E^*$	
Fresh powder	-	-	-	-	$6,08 \pm 0,01$
<i>n</i> -hexane	$-2,17 \pm 0,21$	$2,23 \pm 0,15$	$2,03 \pm 0,20$	$3,72 \pm 0,24$	$5,89 \pm 0,03$
EtOAc	$1,08 \pm 0,28$	$1,86 \pm 0,52$	$2,90 \pm 0,72$	$3,63 \pm 0,80$	$5,15 \pm 0,05$
MeOH	$-1,25 \pm 0,45$	$-2,53 \pm 0,60$	$-3,64 \pm 1,20$	$4,61 \pm 1,39$	$6,35 \pm 0,01$
Hot-water	$-6,28 \pm 0,16$	$-0,72 \pm 0,43$	$-1,66 \pm 0,90$	$6,59 \pm 0,18$	$6,69 \pm 0,04$
NaOH 1%	$-1,11 \pm 1,08$	$-3,84 \pm 0,90$	$-5,18 \pm 2,34$	$6,58 \pm 2,57$	-

Table 2 showed that the extractives determine the bark color, and extractions with different polarity solvents contribute to the color change. After MeOH extraction, the bark showed the highest decrease in redness ( $a^*$ ) and yellowness ( $b^*$ ) values. This result showed that a large concentration of colored compounds was extracted with MeOH. Also, based on the color identification, the extract also showed a darker reddish-brown color compared to other extracts, as shown in Table 1.

On the contrary, extraction using less polar solvents increased the  $a^*$  and  $b^*$  values of the bark. This could be as a result of the color change of the extractives towards red or yellow after extraction while still attached to the bark. A similar trend was also reported by Lukmandaru (2009) on a black streaked teak wood. Further extraction with hot-water and 1 % NaOH showed the highest  $\Delta E^*$  value. This high  $\Delta E^*$  value in the hot-water extraction was influenced by its high  $\Delta L^*$  value. In general, the values of  $\Delta E^*$  of all fractions are greater than 3. According to Hon and Minemura (2001), these findings showed that sappan wood (*Caesalpinia sappan*) bark colors change after extraction could be identified with the naked eyes.



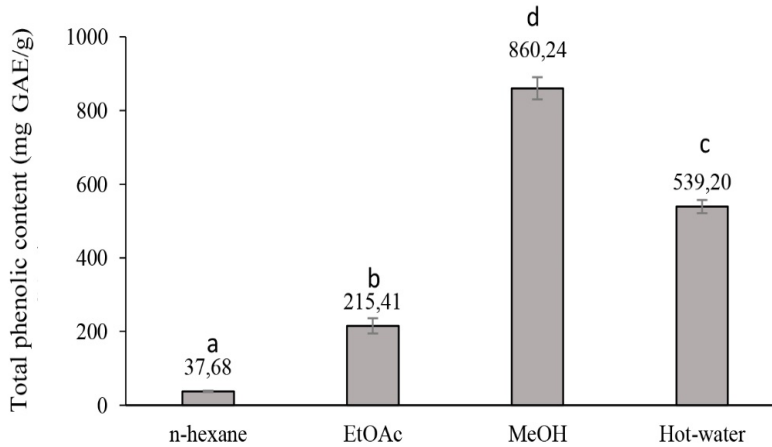
**Figure 1:**  $L^*$ ,  $a^*$ , and  $b^*$  value of sappan wood (*Caesalpinia sappan*) bark after successive extractions.

Polymeric cell walls components and some extractives which form matrix with cellulose, hemicellulose, or lignin could not be extracted with natural solvents. However, extraction with 1 % NaOH has the capacity of breaking some of these matrices, especially hemicellulose, thereby removing its components (Longue *et al.* 2010). Then, the 1 % NaOH extraction reduced the higher value of  $a^*$  and  $b^*$  compared with the natural solvents used in previous extractions. This result shows that some insoluble extractives are still tightly bound to the cell walls matrix of the bark and their contribution to the color very high. This result is in line with the study conducted by Chen *et al.* (2014), where noticeable change in color was reported in pine wood flour after delignification.

### Total phenolic content

The total phenolic content (TPC) levels of each extract are shown in Figure 2. All the extracts gave readable results on TPC measurement. The One-way ANOVA showed a significant difference in the TPC values of extractives with different solvents, at  $p < 0,01$ . Based on Tukey test, the MeOH extract showed the significantly highest at  $860,24 \pm 30,19$  mg GAE/g, followed by hot-water extract at  $539,20 \pm 18,04$  mg GAE/g, EtOAc at  $215 \pm 20,72$  mg GAE/g, while *n*-hexane extract showed the lowest TPC at  $37,68 \pm 1,77$  mg GAE/g.





**Figure 2:** TPC of each extract. Different letters above the histogram (a, b, c, d) indicate significant difference with Tukey HSD test (5 % significance level).

In general, tropical trees contain a large variety of compounds including phenolics, which influence the color differently (Kilic and Niemz 2010, Moya *et al.* 2012). Each solvent showed significant different results with MeOH extraction showing the highest TPC. Based on previous studies, darker color in wood and its extract are associated with high TPC (Dünisch *et al.* 2010, Moya *et al.* 2012, Wu *et al.* 2021). This is confirmed in the dark color of MeOH and hot-water extracts and the high TPC values.

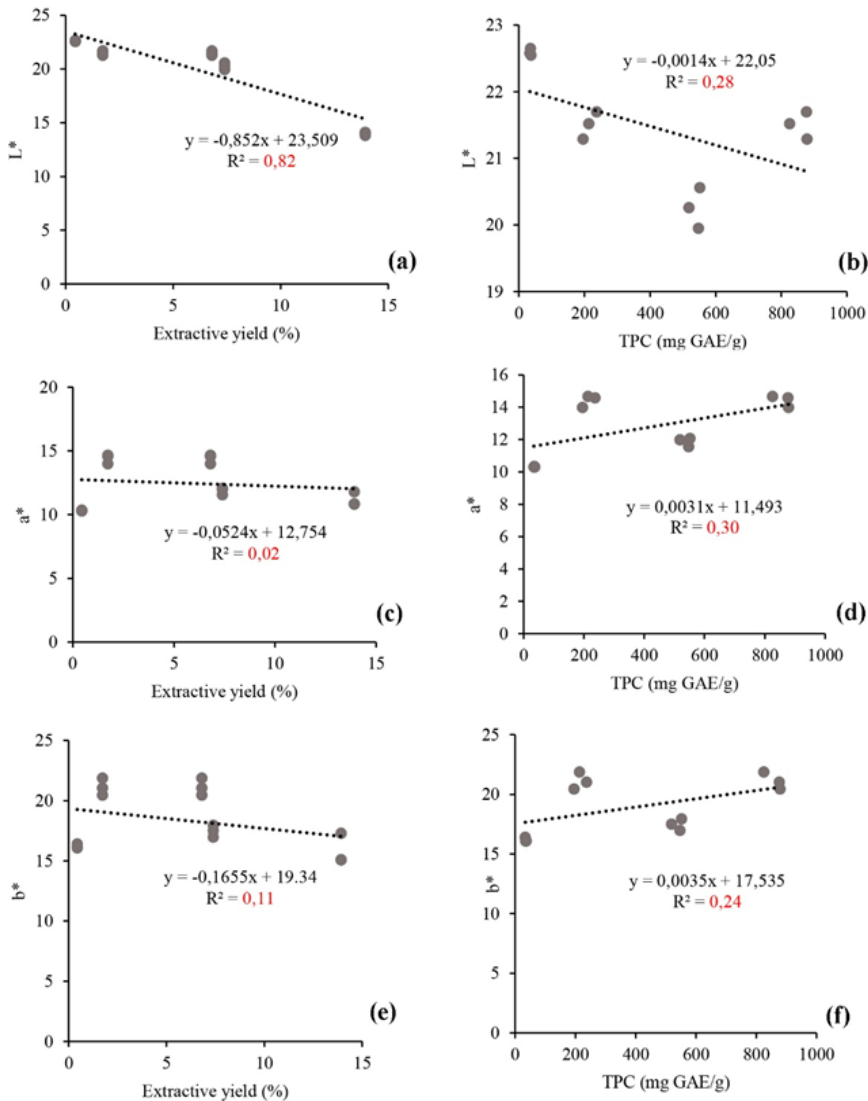
Also, in comparison to a previous work on the bark of different plants, sappan wood (*Caesalpinia sappan*) bark was reported rich in phenolics (Olajuyigbe and Afolayan 2011, Phuyal *et al.* 2020, Negi *et al.* 2012). Similarly, high amount of TPC has also been reported from sappan wood (*Caesalpinia sappan*) heartwood, although slightly lower than the bark from this research (Febriyenti *et al.* 2018).

### Correlation of yield, TPC, and bark color parameters

CIELab color space of the bark before extraction were correlated with its extractive yield and TPC of its corresponding solvent. The coefficient of correlation between yield, TPC, and color parameters are detailed in Figure 3a, Figure 3b, Figure 3c, Figure 3d, Figure 3e, Figure 3f). All correlation of extraction yield and color parameters showed negative interactions. The highest degree of correlation of extraction yield was found with  $L^*$  ( $R^2=0,82$ ), as shown in Figure 3a. Also, the TPC showed a negative correlation with  $L^*$  ( $R^2=0,28$ ), as shown in Figure 3b, though a positive interaction with  $a^*$  ( $R^2=0,30$ ), as shown in Figure 3d, and  $b^*$  ( $R^2=0,24$ ), as shown in Figure 3f).

In this study, a strong negative correlation was found between extractive yield and  $L^*$  value of the bark. Higher correlation between extractive yield and color parameters could indicate that the color change was attributed to the high number of extractives removed from the bark. Specifically, the removal of extractives had the biggest influence on reducing the brightness value. Moreover, negative correlation between the extractive yield and  $L^*$  were previously reported in studies on teak (*Tectona grandis*) (Lukmandaru 2009), cozolmeca (*Vochysia guatemalensis*), and silver wattle (*Acacia mangium*) (Moya *et al.* 2012).

A negative correlation was also observed between the TPC and  $L^*$  showing that phenolics were also responsible in reducing the brightness of the bark. This correlation of TPC and  $L^*$  coincides with previous study on silver wattle (*Acacia mangium*) (Moya *et al.* 2012). In contrast, positive interactions with  $a^*$  and  $b^*$  show that phenolics also influence the bark color towards red and yellow.



**Figure 3:** Correlation between TPC and extractive yield to bark color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ).

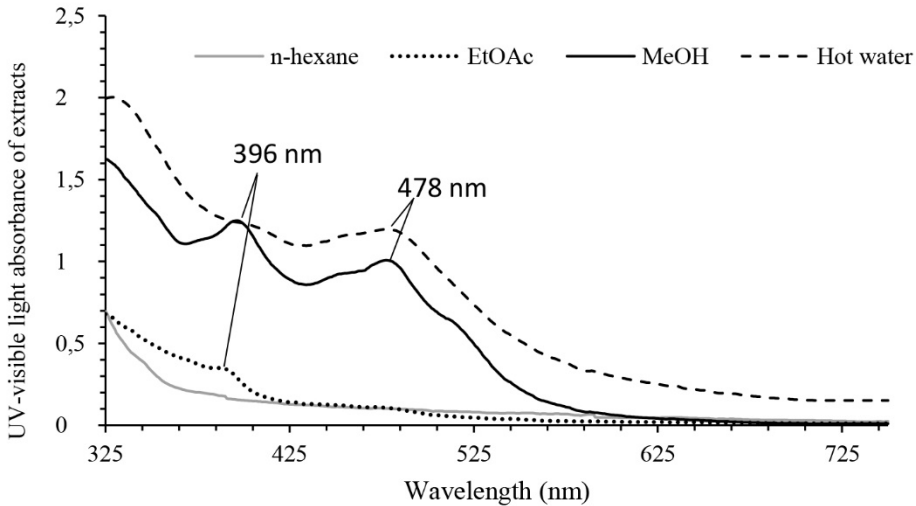
### UV-Vis light absorbance

The absorbances of each *n*-hexane to hot-water soluble extracts are shown in Figure 4. At a concentration of 250 ppm, the hot-water soluble extract showed the highest absorbance. A shoulder was observed in visible light of hot-water extract at 478 nm. However, two peaks were observed at 396 nm and 478 nm in methanol extract. In addition, a small peak was also observed in EtOAc extract at 396 nm, while no visible light peak was observed in the *n*-hexane extract.

In general, the absorbances of the extracts showed a unique pattern with two main peaks at 396 nm and 478 nm. The compounds absorbing light at 396 nm were extracted in MeOH, while a very small fraction was extracted in EtOAc. Additionally, a large fraction of compounds absorbed light at 478 nm in both MeOH and hot-water extracts. The two shoulders at MeOH extract could be the reason for its darker color and higher color change in the bark after extraction, while the single shoulder at hot-water extract could be the reason for its red color. These findings show that the coloration of polar extract might be reason of exhibiting multiple compounds.



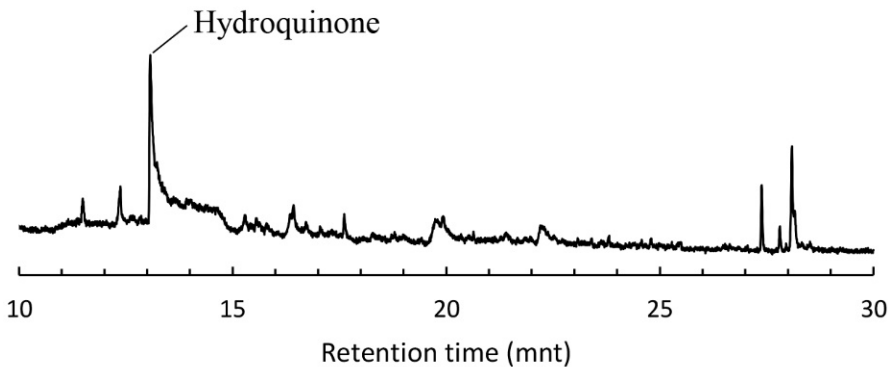
The polar extracts of sappan wood (*Caesalpinia sappan*) bark might also contain brazilin, a major compound isolated and identified from its heartwood which has been known to be a precursor to the red colored dye compound from sappan wood (*Caesalpinia sappan*) (Rosanti *et al.* 2023). A previous research by Zulenda *et al.* (2018) showed that brazilin had an absorbance at 435 nm, but the presence of complexes such as metals could result in bathochromic shift, whereby its light absorbance shifted into longer wavelength.



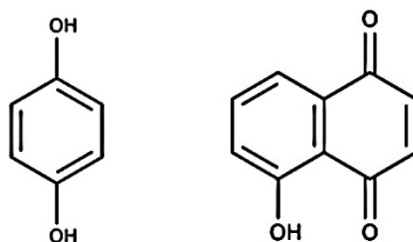
**Figure 4:** UV-visible light absorption of sappan wood (*Caesalpinia sappan*) extracts.

#### GC-MS analysis

The GC-MS analysis of the methanol extract of sappan wood (*Caesalpinia sappan*) showed the presence of a compound similar to hydroquinone, as shown in Figure 5. Hydroquinone is a colorless compound, but this finding shows that the sappan wood (*Caesalpinia sappan*) contains compounds which possess hydroquinone as its precursor.



**Figure 5:** Chromatogram of sappan wood (*Caesalpinia sappan*) bark methanol extract. Hydroquinone was detected at 13,07 min retention time (SI: 86).



**Figure 6:** Structure of hydroquinone (left) and 5-hydroxy-1,4-naphthoquinone (right).

There is a similarity between hydroquinone and a compound previously isolated from sappan wood (*Caesalpinia sappan*) heartwood, 5-hydroxy-1,4-naphthoquinone, in para position of their functional group, as shown in Figure 6 (Lim *et al.* 2005). Naphthoquinone is also a compound with red-brown color, an example of this is 2-hydroxy, 1,4-naphthoquinone, a coloring matter found in henna (*Lawsonia inermis*), also used as a traditional dyeing agent (Bechtold 2009). Furthermore, the UV-Vis spectra maximum absorbance of 1,2-naphthoquinone, and its isomer were reported at 396 nm in methanol solvent (Kuboyama and Matsumoto 1979, Taniguchi and Lindsey 2018).

Based on literatures, it is suspected that quinone type including naphthoquinone compound might also contribute to the color of sappan wood (*Caesalpinia sappan*) bark, along with brazilin and brazilin. Previous studies also suspected the role of quinone on the color of teak (*Tectona grandis*) and *Dyospiros* spp. heartwood (Lukmandaru 2009, Yazaki *et al.* 2015). However, there is need for further studies on isolation and identification of compounds in sappan wood (*Caesalpinia sappan*) bark responsible for its color.

## CONCLUSIONS

Study on the contribution of extractives to *C. sappan* bark color was conducted. The results showed that MeOH extraction had the highest effect on the  $a^*$  and  $b^*$  value. Positive interactions with  $a^*$  and  $b^*$  show that phenolics also affect the bark color towards redness and yellowness values.

The highest extractive yield was obtained with polar solvents such as MeOH and hot-water. By visual, the deepest red color, which described as Tomato Red by RAL, was obtained in the MeOH extract. Due to high TPC value as well as high hydroquinone content by GC-MS analysis, we concluded that coloring matter in the MeOH extract was dominated by phenolic compounds with hydroquinone as its precursor. The UV-Vis analysis observed two shoulders at 396 nm and 478 nm in EtOAc and hot-water extracts respectively, indicating that the color of *C. sappan* bark might be expressed by two major compounds.

Therefore, it suggests that the MeOH extract had the highest contribution to the color of *C. sappan* bark as well as a potential source for natural dye.

### Authorship contributions

- B. A. V. P.: Conceptualization, investigation, methodology, writing - original draft.
- M.: Conceptualization, investigation, methodology, writing - original draft.
- R. P.: Resources, project administration, supervision, writing - review & editing.
- G. L.: Conceptualization, methodology, resources, project administration, supervision, writing - review & editing.

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