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Parametric Study and Characterization of Sappan Wood (*Caesalpinia Sappan Linn*) Natural Red Colorant Extract with Ultrasonic Assisted Extraction Method

***Yuyun Yuniati*[*,1]**
***Dhiya Dini Azmi*[2]**
***Eric Nurandriea*[2]**
***Lailatul Qadariyah*[2]**
***Mahfud*[2]**
[1]*Department of Chemistry, Ma Chung University, Malang 65151, Indonesia*
[2]*Department of Chemical Engineering, Sepuluh Nopember Institute of Technology (ITS), Surabaya 60111, Indonesia*
[*]e-mail: yuyun.yuniati@machung.ac.id

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Abstract. In this study, the optimization and characterization of the colorant extraction process from sappan wood were carried out using the ultrasound-assisted extraction method. Sappan is proposed as a source of natural material wealth, producing a natural red colorant that is being needed by the community in the food sector. Based on this research, the optimum operating conditions for the extraction of sappan wood were obtained using a frequency of 40 kHz, a temperature of 60 °C, a ratio of 0.0050 g mL^{-1}, an extraction time of 20 min, and the use of 60% ethanol solvent. Sappan wood extract created a yellow to reddish orange color at acidic pH (2-6), red at neutral pH (7) and shifted towards purplish red with increasing pH. The extract was qualitatively positive containing quinone, flavonoid, quinone, and tannin compounds, as well as several phenolic compounds detected in the Gas Chromatography Mass Spectroscopy qualitative test.

Keywords: Optimization**,** Sappan Wood, Natural Red Colorant, Ultrasound-Assisted Extraction

INTRODUCTION

Heretofore, coloring is still an essential procedure carried out in the manufacturing process, especially in food or beverage products (Iorizzo et al. 2020, Wörfel et al. 2022). In addition to being used as an aesthetic to attract consumers, the addition of colorant in food is also strengthening the identity of food products, as well as shielding natural bioactive compounds from food ingredients from exposure and damage (De Mejia et al. 2020, Jadhav et al. 2020). The utilization of synthetic dyes is still widely discussed, considering that there has been a lot of misuse of materials, including citizen misconceptions about the maximum limit for the use of materials, the use of non-food grade materials to color food ingredients, as well as the discovery of heavy metal residues in colorant product, thus becoming a serious threat to the society itself (Amchova et al. 2015, Ghosh et al. 2017). With the continuous increase in people awareness and health

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concerns, the selection of natural colorant is increasingly being promoted as edible ingredient that can provide nutraceutical benefits for consumers. Therefore, exploration of various potential natural resources producing natural colorant is perennially conducted. (Yuyun Yuniati et al. 2021, Mohamad et al. 2019, Sabuz et al. 2020).

Sappan (*Caesalpinia Sappan* Linn), or referred to "*Kayu Secang*" in Indonesian, is one of the alternative natural resources that can be used as a source of red colorant from the wood (Sari et al. 2022). This traditional plant has been observed for its potential as an ingredient that has antioxidant and anti-inflammatory property and enhances the flow of the circulatory tract (Batubara et al. 2022). The plant has also being studied for the Diabetes Mellitus Type-2 treatment, pharmacologically (Adnan et al. 2022). Sappan wood contains brazilin compounds as the main active pigment and compound, as well as tannins, flavonoids, and phenolic compounds, from protosappanin, methylsappanol, dan sappanchalcone (Sari et al. 2022). With the promising availability of the sappan plant in Indonesia, this plant can be developed as an asset that has efficacy in the medical and economic aspects (Farmasi et al. 2022).

With regard to the extraction process of natural ingredients, the selection of the right method to obtain extracts of good quality and quantity is still being explored (Mahfud et al. 2022). Several extraction methods have been considered, including maceration with solvents, Soxhlet, steam distillation, and superheated water extraction, although they are less effective in time, amount of solvent, and solubility properties (Góméz-Míguez and Heredia, 2004, Umale and Mahanwar, 2012). In recent years, the extraction procedure that utilizes ultrasonic wave energy has been proposed, with the potential to quickly diffuse solvents into the cell matrix of natural materials and highly solvent interactions with bioactive compounds compared to previous conventional one (Yuyun Yuniati et al. 2021, Y. Yuniati et al. 2021). Ultrasonic method is also a promising method that can produce good extract quality by only using an small amount of solvent (Dey and Rathod, 2013). Therefore, this research focused on the use of ultrasonic-assisted extraction as a method in processing the red natural colorant extract of sappan wood.

This research specifically explored the procedure to obtain as much color extract of sappan wood as possible, with reference to the yield percentage obtained. Optimization was carried out by considering the operating conditions of the solvent selection, the ratio of raw materials to solvents, time, temperature, and the frequency of the ultrasonic device. In addition, the characterization of the extract was also investigated by analyzing the results of product resistance tests, qualitative tests, and chromatography. This preliminary study was able to be an initial breakthrough as a form of recommendation for the use of natural materials for food and beverage coloring.

MATERIALS AND METHODS

Materials

In this study, sappan wood sample was obtained from Gunung Kidul, Yogyakarta, Indonesia in the form of powder. Aquadest and ethanol (C_2H_5OH, 96%, Sigma-Aldrich) was used for the extraction process. Sodium chloride (NaCl, 99.5%, Merck), iron (III) chloride ($FeCl_3$, 98%, Merck), isoamyl alcohol ($C_5H_{12}O$, 99%, Merck), hydrochloric acid (HCl, 37%, Sigma-Aldrich), and magnesium metal were needed for the qualitative characterization of the color extract obtained. Sulfuric acid (H_2SO_4, 98%, Sigma-Aldrich), and sodium hydroxide (NaOH, >97%) were required to characterize the pH test of the color extract obtained.

Equipment Design

The series of equipment for the Ultrasound Assisted Extraction (UAE) method consists of an ultrasonic cleaning bath and a reflux device. The device was composed of a three-neck extractor flask and a condenser, as shown in Fig. 1. The ultrasonic cleaning bath used for this research was the KRISBOW model KW1801033 with a voltage specification of 240 V/50 Hz, 100 W power, frequency: low (20 kHz) and high (40 kHz), tank capacity 2.8 L, tank dimensions: length = 23.5 cm, width = 13.5 cm, and height = 10 cm, and outer dimensions: length = 26.5 cm, width = 16.5 cm, and height = 24 cm.

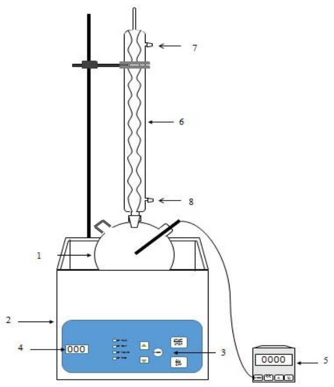


Fig. 1: Equipment arrangement schematic for Ultrasound Assisted Extraction method.

Variable of Process

The optimization carried out in this study varied the use of the ultrasonic cleaning bath frequency: 20 and 40 kHz; temperature: 40, 50, and 60 °C; raw material ratio (mass of sappan dried powder to the solvent volume) were 0.0025, 0.0050, 0.0100, 0.0150, and 0.0200 g mL^{-1}; extraction time: 10, 20, 30, 40 and 50 min; and solvent concentration (20 %; 40%; 60%; 80%; and 96%) ethanol in water solvent.

Extraction

The extraction was done by pre-preparing the sappan dried powder (sieved with a size of 35 mesh) according to the ratio of raw materials to 200 ml of solvent, a series of extraction tools, the extraction process with a predetermined time, temperature, drying of the extract at a temperature of 70-80 °C to obtain the solid weight of the extract.

Yield distribution was carried out on the colorant extract using Eq. (1).

$$\%yield = \frac{mass\ of\ extract\ (g)}{mass\ of\ sample\ (g)} \times 100\% \quad (1)$$

Characterization of Sappan Wood Color Extract

The qualitative test of the extract was carried out by observing the positive and negative content of tannins and flavonoids using the ingredients mentioned in the previous sub.

The characterization of the colorant extract pH test was carried out by dissolving 0.1 gram of the extract in H_2SO_4 or NaOH solution until obtained the color changes of the extract that occurred at pH 4, 5, 6, 7, 9, and 10. The concentrations of H_2SO_4 and NaOH used were 1 N and 0.1 N. The amount of volume added depends on the target pH to be achieved.

The content of the colorant extract of sappan wood was analyzed using Gas Chromatography Mass Spectrometry (GC-MS).

RESULTS AND DISCUSSION

Effect of Ethanol Concentration on Extract Yield

The extraction process was carried out with an ultrasonic frequency of 40 kHz, a ratio of 0.005 g mL^{-1}, 20 min of extraction time, and a temperature of 60 °C. This section used a wider range of ethanol concentrations, including 20% to 100%. Based on Fig. 2, it is clear that the extraction of sappan wood produces a relatively constant yield at 40%, 60%, and 80% ethanol concentrations. The highest yield was achieved in 60% ethanol solvent.

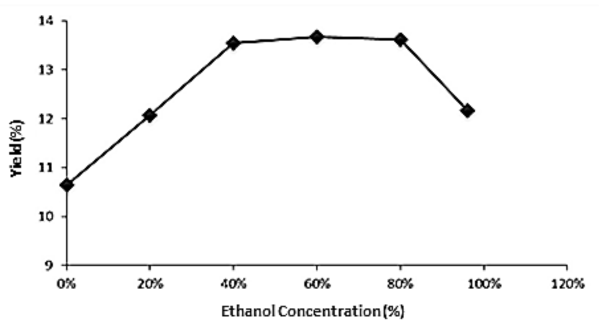


Fig. 2: Yield extract (%) acquisition of natural colorant using the UAE method with different ethanol concentrations.

From top-three different set of solvent concentrations data shown on the chart, the largest percentage yield value was obtained from 60% ethanol followed by 96% ethanol and distilled water for almost all observation points of extraction time. The use of 60% ethanol is a suitable solvent composition to obtain polar and non-polar components from sappan wood. Through this study, the selection of solvent is included as the important factor in the extraction process using the Ultrasound Assisted Extraction (UAE) method. Brazilin, a pigment compound in sappan wood, is a compound that has a similar polarity level to ethanol, with a number of hydroxy groups in its chemical structure (Putri et al. 2018).

Increasing the ethanol concentrations of 40% to 80% resulting in relatively similar yields. However, when the ethanol concentration was increased above 80% the yield obtained decreased. This was due to the possibility that ethanol dissolves in the non-polar coloring compounds found in sappan wood (Smith and Snyder, 2005). It should be noticed that the sappan wood also contains several non-polar compounds that have the potential to reduce extraction yields, such as choumarin and chalcone (Nirmal et al. 2015).

Effect of Extraction Time and Solvent Type Selection on Extract Yield

At this stage, the extraction used water, 60% ethanol and 96% ethanol as solvents. The ultrasonic wave frequency used was 40 KHz. The ratio used in this study was 0.005 g mL^{-1}. The extraction of natural colorant from sappan wood using distilled water, 60% ethanol and 96% ethanol was carried out at a temperature of 60 °C. Extraction time was carried out for 10, 20, 30, 40, and 50 min. The resulting yield is illustrated in Fig. 3.

The result shows that there was an increase in the extraction yield until the system reached the optimum condition. In this study, the optimum extraction result was achieved at 20 min extraction. At that extraction time, the increase of yield percentage corresponds to the initial duration of exposure to ultrasonic waves that would increase swelling and hydration of the sappan wood solid material due to the cavitation effect. The cavitation effect induces the formation of microjets on the surface of the material which can damage the material and cause the solvent to easily diffuse into the matrix of the wood material. In these conditions, the ability of the solvent to obtain the colorant was increasing so that the yield of the extract was getting bigger.

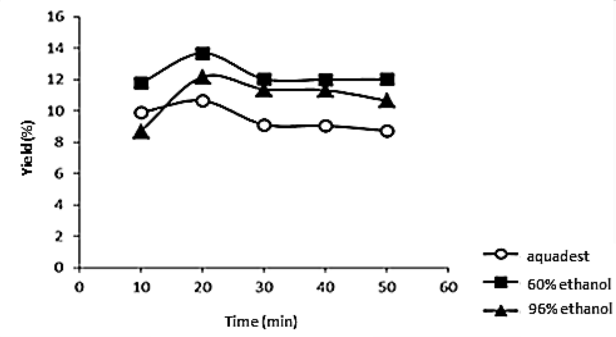


Fig. 3: Differences in yield extract due to variations in extraction time, with the value of the ultrasonic wave ratio and frequency kept constant at 0.005 g mL^{-1} and 40 kHz.

Effect of Frequency Affected on Extract Yield

In the first part of the study, extraction from sappan wood was observed by the frequency level of Ultrasound Assisted Extraction (UAE). The ultrasonic cleaning bath only used two variations of frequency selection: 20 kHz and 40 kHz. The extraction process was carried out using 60% ethanol as a solvent, a raw material and solvent ratio of 0.005 g mL^{-1}, the extraction temperature of 60 °C, and the extraction time of 20 min.

In this study, the yield percentage obtained was 13.2888% (20 kHz) and 13.6763% (40 kHz). Tere was a difference in the yield of sappan wood extract by 2.9160% using the two different frequencies. The bursting of cavitation bubbles is affected by the frequency of the ultrasonic waves. The increase in ultrasonic wave frequency is inversely proportional to the size of the cavitation bubble. The greater the frequency of the ultrasonic waves, the smaller the size of the cavitation bubbles so that the bubble bursting time is faster. The bursting temperature of the cavitation bubble will increase as the ultrasonic wave frequency increases. The thermal effect resulting from an increase in temperature plays a role in the process of destroying plant cell walls so that it can increase extraction yields.

Effect of Temperature on Extract Yield

In this study, natural colorant was extracted from the raw material of sappan wood using the Ultrasound Assisted Extraction (UAE) method. The extraction process was carried out by exposure to ultrasonic waves originating from the ultrasonic cleaning bath. Extraction of pigment was carried out at an ultrasonic wave frequency of 40 kHz. The ratio used in this study was 0.005 g mL^{-1} using distilled water, 60% ethanol and 96% as solvent. Variations of extraction temperature are 40, 50, and 60 °C.

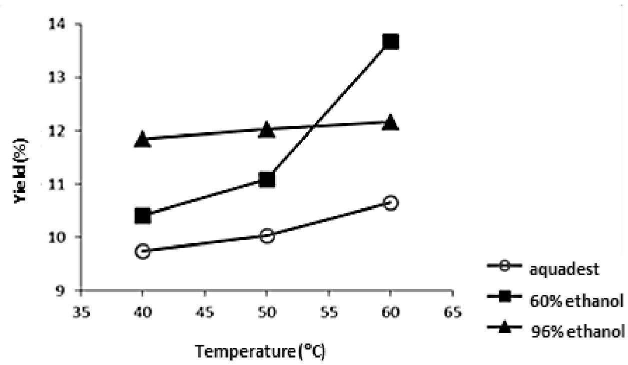


Fig. 4: Differences in yield extract due to variations in temperature, with the value of the ultrasonic wave ratio, extraction time, and frequency kept constant at 0.005 g mL^{-1}, 20 min, and 40 kHz.

Based on Fig. 4, the sappan wood extraction produced the highest extraction yield at temperature of 60 °C. The extraction temperature has a great influence on the UAE process. Increasing the temperature affects the decrease in viscosity and surface tension of the solvent so that it can reduce the cavitation threshold value. The lower the cavitation threshold, the easier it is for cavitation bubbles to form. The bursting of cavitation bubbles can cause damage to plant cell walls. This damage causes the solvent to diffuse into the plant matrix and extract the colorant compounds, thus the extract yield increases more easily.

Effect of Raw Material Ratio on Extract Yield

In this study, natural colorant was extracted from the raw material of sappan wood using the Ultrasound Assisted Extraction (UAE) method. The natural colorant extraction process was carried out by exposure to ultrasonic waves originating from the ultrasonic cleaning bath. Extraction was carried out at an ultrasonic wave frequency of 40 KHz. The ratios used in this study were 0.0025 g mL^{-1}, 0.0050 g mL^{-1}, 0.0100 g mL^{-1}, 0.0150 g mL^{-1} and 0.0200 g mL^{-1}. Extraction from sappan wood was using 60% ethanol solvent at a temperature of 60 °C with an extraction time of 20 min.

Based on the results presented in Fig. 5, it is shown that the optimum ratio for the sappan wood extraction was 0.0050 g mL^{-1}. There was an increase in the extraction yield from the ratio of 0.0025 g mL^{-1} to 0.0050 g mL^{-1}. In addition, there was a decrease in the extraction yield with the increasing ratio of raw materials. The increase in extraction yield occurred because of the increase in the amount of raw material in the solvent. The decrease in extraction yield at the ratio of 0.0100 g mL^{-1} and 0.0150 g mL^{-1} occurred due to an increase in the amount of material while the solvent volume remained. The larger mass of material with constant volume of ethanol promotes the decreasing of the ability to dissolve ethanol against dyes. The highest yield was obtained when the ratio of material to ethanol was 0.005 g mL^{-1}.

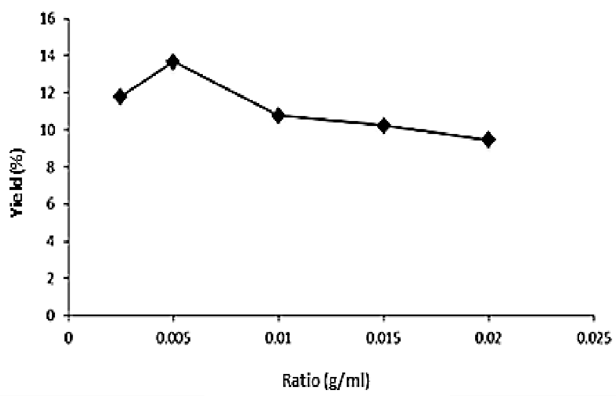


Fig. 5: Yield extracts acquisition of colorant extract based on the differences of raw material ratio with 40 kHz of ultrasonic wave frequency and ethanol (60%) solvent

Increasing the ratio of raw materials to solvents affected the viscosity of the solvent to increase and thereby, preventing the formation of cavitation. The cavitation effect is the most important part of the Ultrasound Assisted Extraction method because it can damage plant cell walls and increase the ability of solvent penetration into plant material to the targeted colorant compound. Higher solvent viscosity will increase the cohesive force of the solvent so that the negative pressure decreases, preventing cavitation.

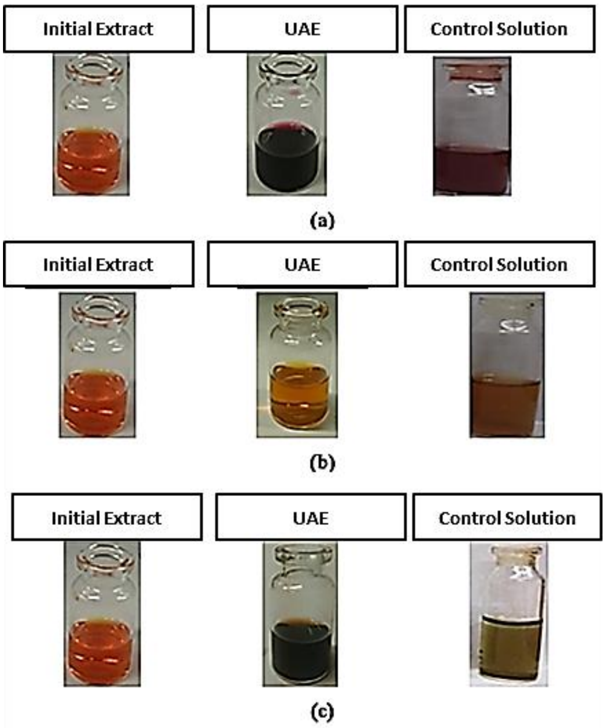
Qualitative Test of Extracts Against the Detection of Quinone, Flavonoid, Quinone, and Tannin Compounds

Qualitative testing aimed to predict colorant compounds extracted from sappan wood. Qualitative testing was done by adding an identification reagent to the colorant solution to prove the presence of flavonoid compounds, tannins, and quinones or other compounds of interest.

Qualitative test of quinone on sappan wood extract is presented in Fig. 6(a). When the sappan wood extract was added with 1% NaOH solution, there was a color change from reddish to red color of the sample with UAE and control solution.

In the analysis of the content of flavonoid compounds contained in the sappan wood extract as presented in Fig. 6(b), a control solution of quercetin, which is a flavonoid derivative compound, was used. In the analytical procedure, magnesium ion powder (99%) and HCl solution (1 N) were used. When the sample was added with HCl, there was a color change from the light orange to dark orange for the sample with UAE and control solution. With this color change, the tested sappan wood extract was confirmed to contain flavonoid compounds because the color change that occurred was the same as or close to the color change of quercetin as a control solution. In the process, magnesium metal and hydrochloric acid reacted with the substituents contained in ring A and ring B of the flavonoid structure. The presence of –OH and $–OCH_3$ groups will give a color change.

In the analysis of the presence of tannin compounds contained in the sappan wood extract, as presented in Fig. 6(c), a control solution of tannic acid, which is a flavonoid derivative compound, was used. In the analysis procedure, $FeCl_3$ solution was used. If there was tannin compound, there would be a color change from the standard color, from reddish orange to blackish green. Phytochemical test using $FeCl_3$ was used to determine whether the sample contained phenolic compounds (–OH) indicated by a blackish green or dark blue color after being added with $FeCl_3$. This is because tannin compounds are polyphenolic compounds which when added with $FeCl_3$ will form complex compounds in the presence of Fe^{3+} ions. From the results of the analysis, there was a color change from reddish orange to blackish brown in the sample of the extracted sappan wood solution and the control solution. With this color change, the tested sappan wood extract contained tannin compound because the color change that occurred was the same as or close to the color change of tannic acid as a control solution.

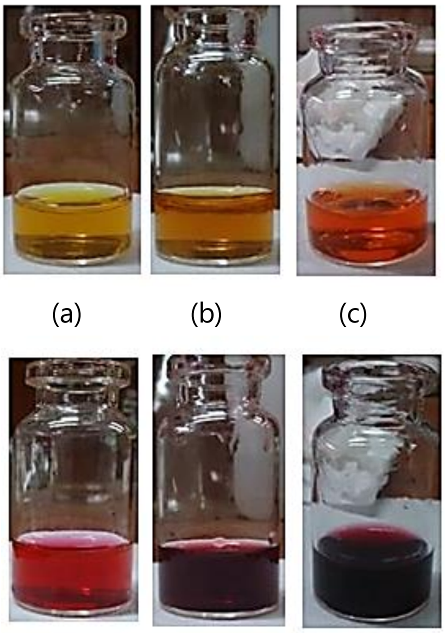


Testing	Reagent	Final Color	Results
Quionens	NaOH	red color	Positive
Flavonoids	Mg+HCl	dark orange	Positive
Tannins	$FeCl_3$	blackish green	Positive

Fig. 6: qualitative test of sappan wood colorant extract on the analysis of: (a) Quinones, (b) Flavonoids and (c) Tannins.

Sappan Wood Red Extract Resistance Test Against pH

Sappan wood 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. At low pH (pH 2-5), brazilein has a yellow color. The brazilein pigment can be applied to foods and beverages that usually have a neutral pH. Based on the experiments that have been carried out, the color change to pH in the extraction of sappan wood is provided in Fig. 7.



(a) (b) (c)

(d) (e) (f)

Fig. 7: Color change of sappan wood extract to pH: (a) pH 4; (b) pH 5; (c) pH 6; (d) pH 7; (e) pH 9; (f) pH 10.

The effect of different pH indicates an increase in absorption (absorbance), with increasing pH (more alkaline). From the color observation of the sappan wood, after increasing the pH to alkaline pH, the color became purplish red. This means that this pigment was unstable at acidic pH.

Characterization of Sappan Wood Red Extract by Gas Chromatography

Through GC-MS analysis, it is possible to know the components of chemical compounds that are predicted to be contained in a natural colorant. Qualitative analysis of sappan wood colorant was carried out by dissolving sappan wood extract in ethanol solvent. The results of GC-MS analysis for sappan wood extract obtained several compounds (Fig. 8), namely phenol, 2-methoxy %area of 5.81; phenol, 3-methoxy with a %area of 6.73; phenol, 2,6-dimethoxy with a % area of 4.06; hexadecanoic acid, methyl ester with an area percentage of 1.51%.

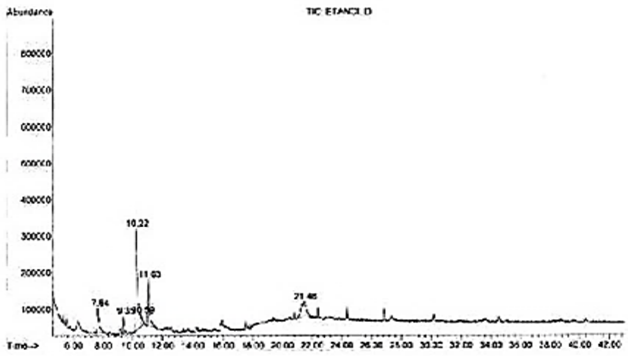


Fig. 8: Chromatogram of sappan wood extract GCMS test results.

CONCLUSIONS

Extraction of natural colorant from sappan wood using the UAE method gave the best results under operating conditions using an ultrasonic wave frequency of 40 kHz, a temperature of 60 °C, a ratio of 0.005 g mL^{-1}, an extraction time of 20 min, and the use of 60% ethanol concentration. Sappan wood extract produced a yellow to reddish orange color on acidic pH testing (2-6), red at neutral pH (7) and shifts towards purplish red with increasing pH. The extract was qualitatively observed to contain quinone, flavonoid, quinone, and tannin compounds, as well as several phenolic compounds detected in the GC-MS test.

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