

Research article

EFFECT OF EXTRACTION SOLVENT ON PHENOLIC AND FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF *CAESALPINIA SAPPAN* L.

Ummi Kalthum Ibrahim^{1,2,✉}, Siti Nur Fairuznisa Pauzi¹, Suzihaque M. U. H.^{1,2}, Nurul Asyikin Md Zaki^{1,2}, Habsah Alwi^{1,2}, Siti Fatma Abd Karim^{1,2}, Noorsuhana Mohd Yusof¹, Syafiza Abd Hashib¹, Rabiatal Adawiyah Abdol Aziz¹ and Nurhaslina Che Radzi¹

¹Faculty of Chemical Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Malaysia

²Food Process & Engineering Research Group (FOPERG), Universiti Teknologi MARA, 40450, Shah Alam, Malaysia

✉Corresponding author: Ummi Kalthum Ibrahim E-mail: ummi985@uitm.edu.my

Mobile: +60127778450, ORCID: 0000-0001-9679-8130

<https://doi.org/10.34302/crpjfst/ASPH5793>

Article history:

Received:

November 25th, 2025

Accepted:

August 25th, 2026

Published

September 20th, 2026

Keywords:

Caesalpinia sappan L.,

Ethanol;

Methanol;

Total phenolic content;

Total flavonoid content;

Antioxidant activity.

ABSTRACT

Caesalpinia sappan L. heartwood contains phenolic and flavonoid compounds with reported antioxidant properties. Solvent selection and extraction conditions can substantially affect the recovery of these compounds. This study evaluated the effects of 70% (v/v) ethanol–water and 70% (v/v) methanol–water on total phenolic content (TPC), total flavonoid content (TFC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of *Caesalpinia sappan* L. heartwood extracts. Response surface methodology (RSM) was used to investigate extraction temperature (30–70 °C) and time (15–45 minutes) and to identify conditions that simultaneously maximize the measured responses. The highest experimentally observed TPC and TFC values were 7.8 and 8.1 mg/g, respectively, while the highest reported DPPH inhibition was 93% for the ethanol extract at 65 °C for 40 minutes. RSM-based optimization predicted a desirability solution for ethanol at 65 °C and 38 minutes, with predicted responses of 4.60 mg/g TPC, 6.80 mg/g TFC, and 85% inhibition; the corresponding methanol solution was 52 °C and 35 minutes, with predicted responses of 1.52 mg/g TPC, 2.35 mg/g TFC, and 74% inhibition.

1.Introduction

Caesalpinia sappan L. or Sappan Wood and Brazilwood Tree is a small tree (6m -15m) from Leguminosae family (NParks, 2021). Sappan wood has a spherical, brownish-green stem and rufous-pubescent branches. Its diameter ranges from 15 to 25 cm. The main active ingredient is Brazilin, although there are also additional compounds like triterpenoids, flavonoids, lipids, steroids, and amino acids. Heartwood of the

Caesalpinia sappan L. is a component in traditional medicines (Vij et al., 2023).

Karneng (2024) stated that the community has utilized the *Caesalpinia sappan* L. as a natural remedy to treat a variety of illnesses, including tetanus, blood clots, and pain. It is believed to have potential as a plant with plenty of antioxidant components. In Southeast Asia, sappan wood has also been used to colour foods like rice and noodles, produce fine furniture and decorative goods, and dye textiles made of silk

and cotton. Anthraquinones, phenolic acids, and flavonoids are the main components of *Caesalpinia sappan* L. (Vij et al., 2023). Xanthonenes, coumarins, chalcones, flavones, and brazilin are among the phytochemical components found in *Caesalpinia sappan* L. or Secang wood (Koeswantika Putri & Indah Sabila, 2021). In recent years, the food, pharmaceutical, cosmetic, and functional food industries have mostly used natural sources to produce bioactive chemicals. Phytochemical substances found in medicinal plants are abundant and have positive health effects. The possible antibacterial, anti-inflammatory, and antioxidant properties of *Caesalpinia sappan* L. extracts have been the main topic of numerous research (Insuan et al., 2024). Sappan wood thus is packed with many other biochemicals such as xanthonenes, flavones, chalcones, and brazilin. Brazilin-a principal active element of *Caesalpinia sappan* L. is a white phenolic compound grouped based on its molecular structure into bioflavonoids which is characterized by two aromatic rings (Vij, 2023).

The phenolic compounds in sappan wood are very beneficial for human health. Phenolic compounds can provide good health by reducing the metabolic disorders. Rahman et al. (2022) states that "Many studies have indicated that phenolic compounds have diverse effects of anti-inflammatory, anti-aging, and antioxidant properties". Phenolic compounds are a diverse group of secondary metabolites formed during the metabolism of plants. Many experts focus on phenol compounds for the reason of health benefits, particularly those found in food. Vitamins C and E are the most often utilized antioxidant supplements. Compared to vitamin C and vitamin E, flavonoids have a stronger antioxidant capacity. Bone health is improved by flavonoids because of their well-known and enhanced antioxidant and anti-inflammatory effects. There are many opportunities for bone tissue engineering when flavonoids are included in biomaterials. Flavonoids, which may be used as sunscreens, are found in the secang tree. Conjugated aromatic benzene groups in flavonoid compounds could absorb ultraviolet

(UV) light and are thought to protect skin exposed to direct sunlight (Rohmah et al., 2022).

Brazilin is the main compounds in Sappan wood that has numerous industrial uses in the medicinal, cosmetic, and textile sectors (Vij et al., 2023). According to Vij (2023), Potential pharmacological activities of brazilin include immunostimulant, anti-inflammatory, anti-tumor, and anti-diabetic effects. Brazilin can bind iron to both ferrous and ferric ions (Taveepanich et al., 2024). Since brazilin is the primary active component of the flavonoid group, the red dye found in sappan wood is referred to as a brazilin group compound (Koeswantika Putri & Indah Sabila, 2021). The antioxidants could slow, stop, and reverse the lipid oxidation process, which can harm the body's cells. The antioxidants work in the body is by binding free radicals too. The body's regular metabolism produces free radicals, which can lead to oxidative damage, including damage to DNA, membranes, and cells (Koeswantika Putri & Indah Sabila, 2021). Synthetic antioxidants and natural antioxidants are the two categories of antioxidants. When an antioxidant substance is produced via a chemical reaction, it is referred to as a synthetic antioxidant. Antioxidant molecules that are derived from natural components are known as natural antioxidants. Vegetables, seeds, stems, and fruits are typically plants that contain natural antioxidants. Flavonoids and phenolic *Caesalpinia sappan* L. have various benefits in human health, but none are apparently nullified by evidence toward their extraction techniques from *Caesalpinia sappan* L. Moreover, even fewer studies have been carried out on appropriate solvents used to recover these bioactive compounds generated from *Caesalpinia sappan* L. Accordingly, extraction solvents are an important part in the success rate of bioactive compound extraction from plant products, especially from *Caesalpinia sappan* L.

A successful extraction process depends on factors such as the method applied, solvent, and the nature of the target compounds. Conventional techniques like maceration and Soxhlet extraction have been in use, also modern extraction techniques such as Ultrasound-

Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) might be more efficient. The extraction efficiency depends on the type of the solvent used so that the bioactive compound is not lost or destroyed during the extraction process. The solvent system used is mostly determined by the bioactive molecule being targeted. Various solvent systems are available to extract bioactive compounds from natural sources. Hydrophilic chemicals are extracted using polar solvents such as methanol, ethanol, or ethyl acetate (Sasidharan et al., 2011). Ethanol has been widely used for the extraction of phenolic compounds because it is suitable, nontoxic for researchers and environment. Methanol also expresses highest selectivity towards phenolic compounds (Pintac et al., 2018).

Response surface methodology (RSM) is used as a substitute procedure to lessen the difficulties associated with conventional testing and to assess the impact of several factors. RSM is an effective mathematical and statistical method for examining empirical models that explain how independent factors and their interactions affect response variables (Mansour et al., 2023). As stated by Maccarronello et al. (2024), RSM will enhance the recovery of bioactive chemicals by determining the optimal operating conditions. Compared to the conventional one-factor at a time method, RSM saves time and money by reducing the number of experiments needed for the study. RSM has previously been used to optimize the extraction of polyphenols from a variety of plant-based foods and their byproducts. The objectives of this study are to determine the total flavonoid content, total phenolic content and antioxidant activities in Brazilin extracted from *Caesalpinia sappan* L. using different types of solvent extraction and to compare the effectiveness of different solvents on the total flavonoid content, total phenolic content and antioxidant activities in Brazilin extracted from *Caesalpinia sappan* L.

2. Materials and Methods

2.1. Materials and Sample Preparation

The materials and reagents included *Caesalpinia sappan* L. heartwood, ethanol, methanol, distilled water, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), dimethyl sulfoxide, aluminium chloride (AlCl_3), quercetin, potassium acetate, diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), and laboratory-grade consumables. The instruments included a rotary evaporator, UV–Vis spectrophotometer (GENESYS), water bath, hot plate with magnetic stirring, grinder, and filtration apparatus.

Caesalpinia sappan L. heartwood was obtained from a local supplier and authenticated before use. The material was washed to remove surface impurities and dried in a hot-air oven at 60 °C. The dried heartwood was ground and sieved to a particle size of 300 µm. The powder was stored in airtight containers at room temperature and protected from light until extraction.

2.2. Experimental Design and Response Surface Methodology (RSM)

Response surface methodology (RSM) was employed to investigate and optimize the effects of extraction temperature, extraction time, and solvent type on the extraction of bioactive compounds from *Caesalpinia sappan* L. heartwood. A central composite design (CCD) was used for the two quantitative process variables, namely extraction temperature (X_1) and extraction time (X_2), while solvent type (X_3) was incorporated as a categorical factor. The temperature was investigated over the range of 30–70 °C, with a centre point of 50 °C, whereas the extraction time was varied from 15 to 45 min, with a centre point of 30 min. Two extraction solvents, ethanol and methanol, were evaluated as discrete categorical levels.

For the quantitative variables, the actual experimental levels were transformed into coded values of –1, 0, and +1 according to the standard linear transformation used in RSM. Thus, coded levels of –1, 0, and +1 corresponded to 30, 50, and 70 °C for temperature and 15, 30, and 45

min for extraction time, respectively. Solvent type was coded as a categorical variable, with ethanol and methanol represented by the two categorical levels. The numerical coding assigned to the solvents was used only for experimental-design purposes and did not imply an ordered quantitative relationship between the two solvents.

2.3. Solvent preparation of quercetin and gallic acid standard calibration curve

The quercetin calibration curve was prepared following Masturi et al. (2021) with modifications. Briefly, 50 mg of quercetin was dissolved in 50 mL ethanol. Aliquots of 0.50, 1.00, 1.50, 2.00, and 2.50 mL were diluted with distilled water to 10 mL, giving nominal concentrations of 50, 100, 150, 200, and 250 µg/mL. For each standard, 3 mL of quercetin solution was mixed with 3 mL of 2% (w/v) AlCl_3 and 3 mL of 120 mM potassium acetate. The mixtures were incubated in the dark for 30 minutes, and absorbance was measured at 425 nm against a reagent blank.

The gallic acid calibration curve was prepared according to Masturi et al. (2021) with modifications. Gallic acid (50 mg) was dissolved in ethanol to 50 mL. Aliquots of 1.0–4.0 mL were diluted to 10 mL to obtain nominal concentrations of 100, 150, 200, 250, 300, 350, and 400 µg/mL. One millilitre of each standard was mixed with 3 mL Folin–Ciocalteu reagent in a 50 mL volumetric flask and allowed to react for 5 minutes. Subsequently, 8 mL of 7.5% (w/v) Na_2CO_3 was added and the volume was made up with distilled water. The mixture was incubated in the dark at room temperature. Absorbance was measured at 765 nm.

2.4. Solvent Extraction Method

The extraction procedure was adapted from Settharaksa et al. (2019), with modifications to solvent composition, temperature, and extraction time. Five grams of dried *Caesalpinia sappan* L. heartwood powder was mixed with 100 mL of either 70% (v/v) ethanol–water or 70% (v/v) methanol–water, giving a solvent-to-solid ratio of 20 mL/g. The suspension was heated with continuous magnetic stirring at the

temperature and for the duration specified by the RSM design. After extraction, the mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper. The filtrate was stored at 4 °C until analysis.

2.5. Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method described by Masturi et al. (2021), with modifications in the reagent and sample volumes. Briefly, 1 mL of sample extract or gallic acid standard solution was transferred into a volumetric flask, followed by the addition of 3 mL of Folin–Ciocalteu reagent and 30 mL of distilled water. The mixture was thoroughly mixed and allowed to stand for 10 minutes at room temperature. Subsequently, 8 mL of 7.5% (w/v) sodium carbonate solution was added, and distilled water was added to make up the final volume to 50 mL. The resulting solution was mixed thoroughly and incubated in the dark at room temperature for 30 minutes to allow colour development. After incubation for 45 minutes, the absorbance of the reaction mixture was measured at 765 nm using a UV–Vis spectrophotometer. A calibration curve was constructed using gallic acid standard solutions (0 – 100 mg/L), and the total phenolic content of the samples was calculated from the calibration equation.

2.6. Determination of total flavonoid content

The determination of total flavonoid content (TFC) was carried out according to the method of Masturi et al. (2021), with slight modifications in sample and reagent volumes. Briefly, 2 mL of sample extract and 3 mL of quercetin standard solution were separately added to 4 mL of 0.1 mol/L aluminium chloride solution. The mixtures were then combined and diluted with 70% (v/v) ethanol to a final volume of 25 mL. The reaction mixture was thoroughly mixed and allowed to stand for 30 minutes at room temperature to ensure complete complex formation. Subsequently, the absorbance of the solution was measured using a UV–visible spectrophotometer at 425 nm against an appropriate blank.

2.7. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed according to Masturi et al. (2021), with slight modifications in sample preparation and assay conditions. A DPPH working solution was prepared in methanol. Subsequently, 3 mL of DPPH solution was mixed with 4 mL of methanol and incubated in the dark at room temperature for 45 minutes. The absorbance of the resulting solution was measured using a UV-visible spectrophotometer at 517 nm.

For sample preparation, 2 mg of crude extract and quercetin (standard) were separately dissolved in 10 mL of methanol and then transferred into a 100 mL volumetric flask. The solutions were made up to volume with methanol to obtain a stock concentration of 1000 µg/mL. Serial dilutions were prepared to obtain final concentrations of 10, 20, 30, 40, and 50 µg/mL. 3 mL of each sample concentration was mixed with 1 mL of DPPH solution in methanol. The reaction mixtures were incubated in the dark at room temperature for 30 minutes. Methanol containing DPPH without sample was used as the control, while methanol alone served as the blank.

All measurements were performed in triplicate, and the percentage of DPPH radical scavenging activity was calculated using Equation (1).

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (1)$$

where A_{control} is the absorbance of the DPPH control and A_{sample} is the absorbance of the extract-containing reaction mixture after the same incubation period.

3. Results and discussion

The extraction experiments compared 70% (v/v) ethanol–water and 70% (v/v) methanol–water over extraction temperature of 30–70 °C and extraction time of 15–45 minutes.

3.1. Total phenolic content

The response surface plot demonstrates that extraction temperature and time exert a significant combined effect on total phenolic content (TPC) when ethanol is used as the

solvent, as evidenced by the dome-shaped curve (Figure 1). An increase in temperature and extraction time initially enhances TPC, which can be attributed to improved solubility of phenolic compounds in ethanol, reduced solvent viscosity, and increased cell wall permeability that collectively promote mass transfer from the plant matrix into the solvent. However, beyond the optimum region, further increases in temperature and prolonged extraction time result in a decline in TPC, most likely due to thermal degradation, oxidation, or polymerization of heat-sensitive phenolic compounds. The elliptical contour pattern confirms a strong interaction by temperature and time combination, indicating that optimal phenolic recovery is achieved only within a moderate range of both parameters rather than at extreme conditions. These findings are consistent with previous studies reporting that ethanol-based extraction of phenolics is governed by a balance between enhanced diffusion kinetics and compound stability, where excessive thermal exposure negatively affects phenolic integrity (Cacace & Mazza, 2003; Lapornik et al., 2005; Dai & Mumper, 2010).

The highest concentration for TPC obtain is 7.8 mg/ml as shown in Figure 1 by using ethanol as solvent for extraction. For methanol solvent, the highest value of the concentration is 2.8 mg /ml after compared with standard curve based on Figure 2. The range for TPC concentration value is (0.7- 7.8) mg/ml for ethanol and for methanol is (0.08- 2.8) mg/ml. Based on Figure 1 and Figure 2, the colour blue represents the lowest value of concentration obtained for the total phenolic content while the red colour represents the highest concentration value. The experimental value obtained conclude that ethanol extracts more phenolic content compared to methanol consistent with Kurniasari et al. (2024), who reported that ethanol gave the highest phenolic content. This supports the study that ethanol has superior efficacy for extracting phenolic compounds like brazilin due to its polarity and thermal profile.

The extraction time that will give higher concentration of TPC is 40 minutes; however, 45 minutes give lower value of TPC. This means

longer extraction time can result in degradation of phenolic compound resulting in lower TPC value. As stated by Che Sulaiman et al. (2017), at fixed temperature, an increase in extraction time slightly decreased the yield. Also, at 65°C extraction temperature results in the highest

concentration of total phenolic content because theoretically, under high temperatures, plant tissues are softened, and the weak interactions affect the cell membranes. As a result, phenolic compounds can be easily extracted into the solvent (Che Sulaiman et al., 2017).

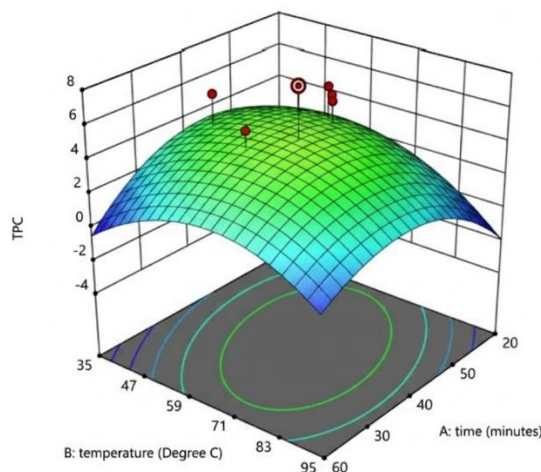


Figure 1. Effect of temperature and time on TPC using ethanol as solvent.

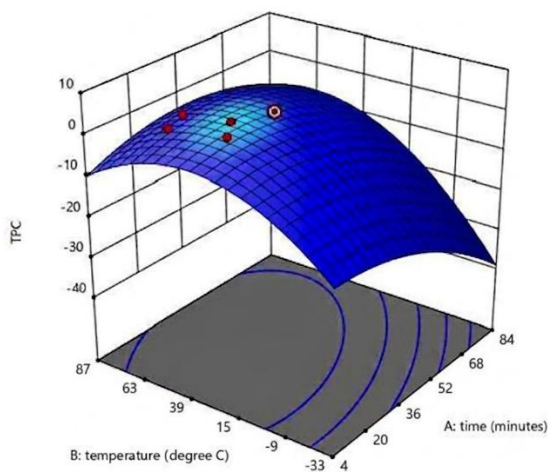


Figure 2. Effect of temperature and time on TPC using methanol as solvent.

To evaluate the adequacy and predictive accuracy of the established response surface methodology (RSM) model for total phenolic content (TPC) extraction using ethanol and methanol systems, the predicted values derived from the model equations were plotted against the experimentally observed actual values (Figure 3). The predicted versus actual plot for total phenolic content (TPC) demonstrates an

overall good agreement between experimental and model-predicted values for both ethanol and methanol extracts, as indicated by the clustering of most data points near the 45° reference line. The diagnostic plot reveals a positive linear progression, indicating that the model successfully captures the overarching upward trend of TPC yields across the experimental design space. However, a detailed analysis of the

residual distribution around the 45-degree line indicates a moderate correlation coefficient ($R^2 = 0.42$). The colour coding represents different sample groups corresponding to extraction. Dark blue points represent the lowest observed TPC extraction yields, meanwhile light blue points indicate intermediate TPC extraction yields. The green points correspond to high TPC extraction yields. The data points associated with ethanol extraction are generally distributed at higher actual and predicted TPC values, reflecting the greater extraction efficiency of ethanol, while methanol data points are concentrated in the lower TPC range. Minor

deviations from the parity line, particularly at lower concentrations, suggest some variability in model prediction, which may be attributed to experimental noise or the complex influence of extraction parameters on phenolic release. Nonetheless, the absence of systematic over- or under-prediction and the random dispersion of residuals indicate that the developed model is robust and adequately captures the relationship between process variables and TPC for both solvents, supporting its suitability for process optimization and prediction within the studied experimental domain.

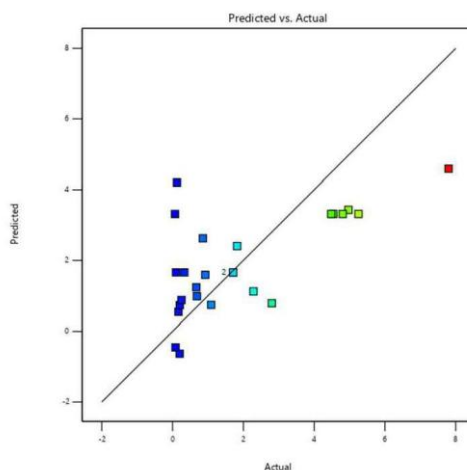


Figure 3. Graph predicted vs actual for the concentration of TPC of both solvent ethanol and methanol.

3.2. Total flavonoid content

Figure 4 illustrates that extraction temperature and time significantly influence total flavonoid content (TFC) when ethanol is used as the solvent, with a clear interaction between both variables. An initial increase in temperature and extraction time leads to a marked rise in TFC, which can be attributed to enhanced solubility of flavonoids in ethanol, improved mass transfer, and greater disruption of plant cell walls that facilitate the release of intracellular compounds. However, beyond the optimal region, further increases in temperature and prolonged extraction time result in a decline in TFC, indicating the susceptibility of flavonoids to thermal degradation, oxidation, and possible structural transformation under excessive heat exposure. The curved response surface and elliptical contour lines confirm that

moderate extraction conditions are more favorable than extreme settings, as excessive thermal energy combined with long extraction durations may accelerate degradation kinetics and reduce flavonoid stability. Overall, the observed trend highlights the balance between improved extraction efficiency and compound stability, emphasizing that optimal temperature–time combinations are critical for maximizing flavonoid recovery while minimizing degradation during ethanol-based extraction.

Compared with the ethanol-based extraction, extraction using methanol as solvent indicates a similar general trend in which total flavonoid content (TFC) initially increases with increasing temperature and extraction time but reaches its optimum at comparatively lower conditions, followed by a more pronounced decline at higher temperatures and prolonged

durations (Figure 5). While ethanol shows a broader optimum region and better flavonoid retention at moderate–high temperature–time combinations, the methanol system appears more sensitive to thermal and time-related effects, as reflected by the steeper curvature of the surface and tighter contour lines. This behavior can be attributed to the higher polarity and volatility of methanol, which enhances rapid solubilization of flavonoids at shorter times but also accelerates degradation, oxidation, or solvent loss under excessive thermal exposure. In contrast, ethanol provides a more stable extraction environment, reducing flavonoid degradation and allowing sustained extraction efficiency over a wider operational range. Overall, the comparison suggests that methanol favors faster extraction under milder conditions, whereas ethanol is more suitable for achieving higher and more stable flavonoid yields when moderate increases in temperature and time are applied.

Total flavonoid content value is the highest at 65°C for 40 minutes of extraction using

ethanol solvent which gives the concentration of 8.1 mg/ml as shown in Figure 4. Based on Figure 5, for methanol solvent, highest concentration is at 30 minutes and 70°C which is 5 mg/ml. From the result, the concentration of TFC for ethanol solvents ranges from 0.14 mg/ml to 8.1 mg/ml while for methanol the concentration ranges from 0.11 mg/ml to 5 mg/ml.

The highest value for the concentration of total phenolic content was obtained when extracted by using ethanol-water mixture. From this, flavonoid content is easier to extract by using ethanol than methanol. Among the solvent extracts, the flavonoid content of the water and ethanol extracts was higher than that of the methanol extract (Seo et al., 2014). Figure 6 shows the predicted versus actual plot for total flavonoid content (TFC) shows a strong agreement between experimental and model-predicted values, as most data points are closely distributed around the 45° reference line, indicating good predictive accuracy of the developed model.

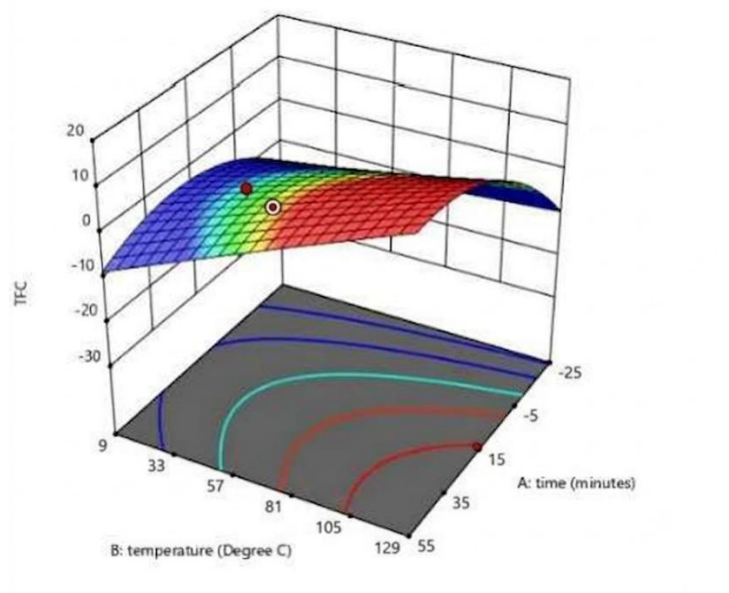


Figure 4. Effect of temperature and time on TFC using ethanol as solvent.

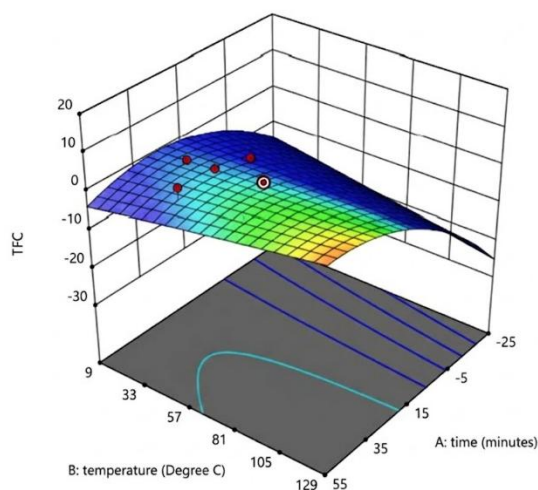


Figure 5. Effect of temperature and time on TFC using methanol as solvent.

This translates to an approximate coefficient of determination, $R^2 = 0.72$, confirming that the model successfully accounts for a substantial majority of the experimental variance driven by the extraction variables. Low concentration regime of TFC represents dark blue data points, meanwhile intermediate regime of TFC represents by green data points. Yellow and red data points show high yield regime of TFC. Data points at lower TFC concentrations are more tightly clustered, suggesting higher model reliability in the low to moderate flavonoid range, while slightly larger deviations are

observed at higher concentrations, which may be attributed to increased experimental variability or nonlinear extraction behavior at elevated temperature–time conditions. Importantly, no systematic over- or under-prediction is evident, as points are symmetrically scattered around the parity line. Overall, the plot confirms that the model adequately captures the relationship between extraction parameters and TFC concentration, supporting its suitability for prediction and optimization of flavonoid extraction within the studied experimental domain.

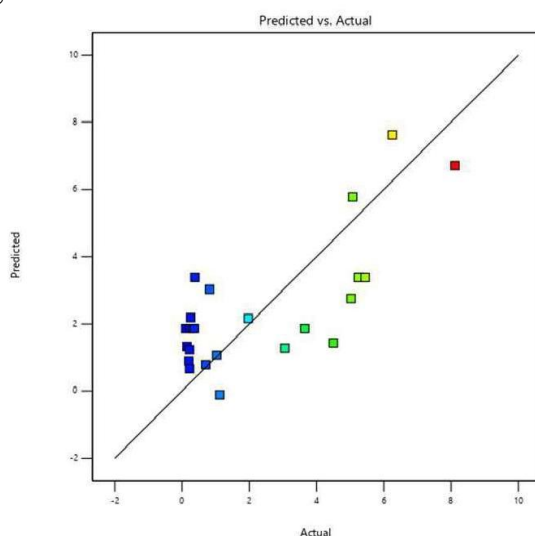


Figure 6. Graph predicted vs actual for the concentration of TFC of both solvent ethanol and methanol.

3.3. Antioxidant activity

Antioxidant activity is being measured by the percentage (%) inhibition calculated from equation (1). From both Figure 7 and Figure 8 the highest percentage inhibition obtained from

solvent ethanol at 65°C for 40 minutes of extraction which is 93% followed by extraction using methanol for 30 minutes at 45°C which is 91%.

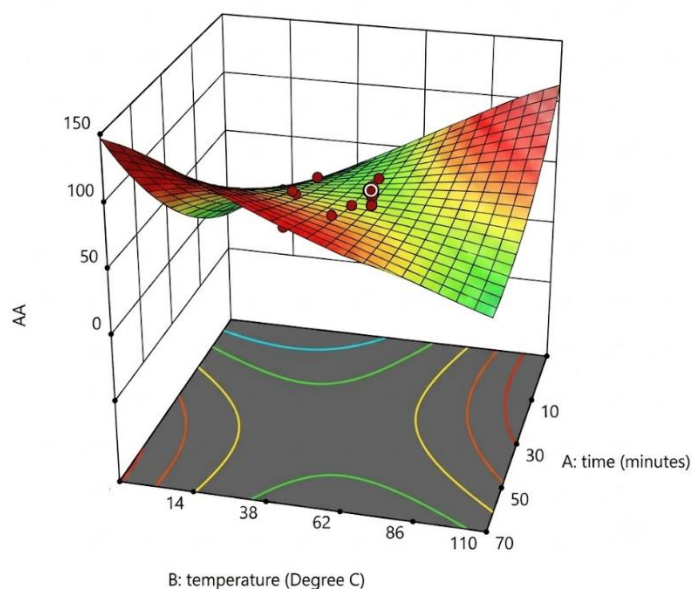


Figure 7. Effect of temperature and time on Antioxidant activity using ethanol as solvent.

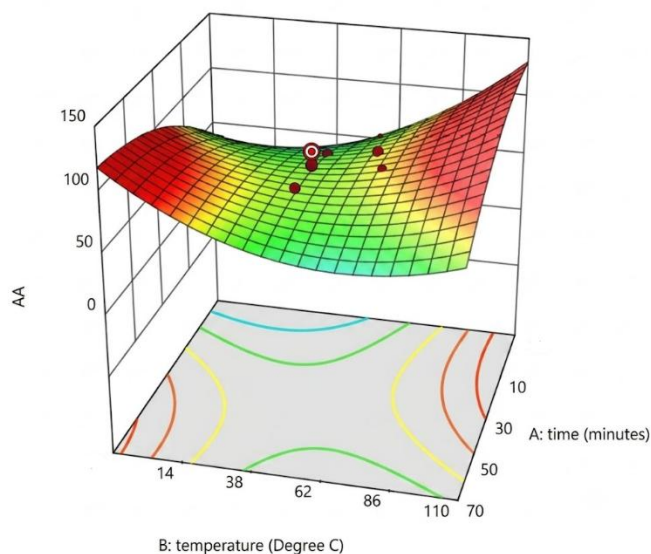


Figure 8. Effect of temperature and time on Antioxidant activity using methanol as solvent.

Antioxidant activity (AA) extracted using ethanol is significantly influenced by both temperature and extraction time, with a strong interaction between the two variables. Antioxidant activity increases markedly as

temperature rises from low to moderate levels, indicating enhanced solubility and extraction efficiency of antioxidant compounds in ethanol. Similarly, increasing extraction time from short to moderate durations leads to a substantial

increase in AA, reflecting improved diffusion and solvent–solute contact. The highest antioxidant activity is observed at moderate to high temperatures (approximately 80–100 °C) combined with moderate extraction times (around 30–40 minutes). However, at very high temperatures and prolonged extraction times, the increase in AA becomes less pronounced or slightly declines, suggesting possible degradation of ethanol-extractable antioxidants. Overall, the figure demonstrates the existence of an optimal temperature–time region for maximizing antioxidant activity when ethanol is used as the solvent. Based on the response surface plot, antioxidant activity extracted using methanol is influenced by both temperature and extraction time, with a clear interaction between these factors. Antioxidant activity increases as temperature and time rise from low levels, indicating enhanced extraction efficiency due to improved solubility and mass transfer of antioxidant compounds in methanol. However, at excessively high temperatures or prolonged extraction times, the antioxidant activity begins to decline, likely due to thermal degradation or oxidation of heat-sensitive compounds. The highest antioxidant activity is achieved at moderate to high temperatures combined with moderate extraction times, suggesting the presence of an optimum extraction condition where maximum antioxidant yield can be obtained without compromising compound stability.

The results show that both methanol and ethanol extracts exhibit a significant dependence of antioxidant activity on temperature and extraction time, with clear interaction effects. In both solvents, antioxidant activity increases from low values at short extraction times and low temperatures, reaching maximum levels at moderate extraction times (≈ 30 – 40 minutes) and elevated temperatures (≈ 80 – 100 °C), indicating that heat and sufficient contact time enhance mass transfer and solubility of antioxidant compounds. However, the methanol system shows a more pronounced decline in antioxidant activity at higher temperatures and longer

extraction times, suggesting greater susceptibility to thermal degradation or solvent–compound interactions. In contrast, ethanol extraction demonstrates a broader high-activity region and a more gradual response at elevated conditions, implying better stability of antioxidants or a milder extraction environment. Overall, while both solvents are effective, ethanol appears to provide a slightly more stable antioxidant response over a wider range of temperature and time compared to methanol.

Figure 9 shows the predicted vs actual graph for the inhibition of antioxidant activity of both solvents. Based on the graph, the distribution of points shows a moderate alignment along the diagonal line, indicating that the model has a fair predictive potential. However, certain points are far above or below the line, showing underprediction or overprediction in some samples. Also, ethanol solvents give the highest value for antioxidant activity. As stated by Ghazzawi et al. (2021), the antioxidant activity increases with increasing of ethanol concentration up to 70% and depended on total phenolic content. In line with this study, which highest TPC obtained is by using ethanol as solvent.

The predicted versus actual plot for antioxidant activity of both ethanol and methanol extracts shows that most data points are closely distributed around the 45° reference line, indicating good agreement between the experimental and model-predicted values. This suggests that the developed models are reliable in predicting antioxidant activity for both solvents within the studied range. Minor scatter of points away from the diagonal line is observed, particularly at lower and higher antioxidant activity values, reflecting small prediction errors that may arise from experimental variability or solvent-specific extraction behavior. Overall, the close alignment between predicted and actual values confirms the adequacy and validity of the models for describing antioxidant activity in both ethanol and methanol extractions.

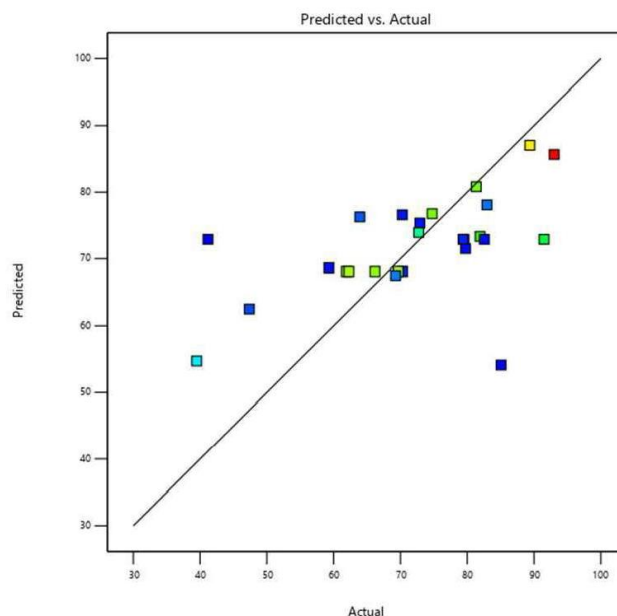


Figure 9. Graph predicted vs actual for the Antioxidant activity of both solvent ethanol and methanol.

Table 1 shows the summary of the final quadratic regression equations and statistical performance metrics for Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity (AA) in terms of actual factors (X_1 = time and X_2 = temperature) across ethanol and methanol solvents. The regression model for TFC demonstrated the highest predictive fit, reaching near-statistical significance ($p = 0.0563$) and explaining 53.68% of the variance ($R^2 = 0.5368$), whereas the models for TPC ($p = 0.2159$, $R^2 = 0.4201$) and AA ($p = 0.5723$, $R^2 = 0.2862$) were statistically

non-significant ($p > 0.05$). Across all three responses, low Lack of Fit values (1.12–1.87) indicate that the second-order model structure is appropriate without severe misspecification. Furthermore, the positive interaction terms (X_1X_2) for TPC and TFC reflect a slight synergistic effect when increasing extraction time and temperature simultaneously, while the negative interaction term for AA points to potential thermal degradation of bioactive compounds during prolonged, high-temperature extractions.

Table 1. Regression Equations and Model Fitting Statistics for TPC, TFC, and AA

Response	Solvent	Regression Equation (X_1 = Time, X_2 = Temperature)	p - value	Lack of Fit	R^2
Total Phenolic Content (TPC)	Ethanol	$Y = -9.055 + 0.108X_1 + 0.352X_2 + 0.003X_1X_2 - 0.004X_1^2 - 0.003X_2^2$	0.2159	1.87	0.4201
	Methanol	$Y = -5.353 + 0.109X_1 + 0.233X_2 + 0.003X_1X_2 - 0.004X_1^2 - 0.003X_2^2$			
Total Flavonoid Content (TFC)	Ethanol	$Y = -6.236 + 0.218X_1 + 0.110X_2 + 0.002X_1X_2 - 0.005X_1^2$	0.0563	1.37	0.5368
	Methanol	$Y = -3.232 + 0.269X_1 + 0.025X_2 + 0.002X_1X_2 - 0.005X_1^2$			
Antioxidant Activity (AA)	Ethanol	$Y = 13.523 + 2.022X_1 + 0.075X_2 - 0.014X_1X_2 - 0.011X_1^2 + 0.010X_2^2$	0.5723	1.12	0.2862
	Methanol	$Y = 54.255 + 1.486X_1 - 0.366X_2 - 0.014X_1X_2 - 0.011X_1^2 + 0.010X_2^2$			

3.4.Optimization

Table 2 shows the validation of the response surface methodology model, comparing predicted and experimental values for Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity under optimized conditions for 70% Ethanol (65°C, 38 minutes) and 70% Methanol (52°C, 35 minutes). Across all responses, the percentage errors remained consistently low between 5.81% and

8.09%, well within the acceptable 10% threshold, confirming the accuracy and reliability of the predictive models. Ethanol consistently outperformed, achieving higher experimental yields for TPC (4.31 ± 0.12), TFC (6.29 ± 0.18), and Antioxidant Activity (79.8 ± 1.5), which demonstrates that a higher temperature and longer duration in an ethanol system optimize the recovery of these bioactive compounds.

Table 2. Predicted and Experimental Values at Optimization Condition

Target Response	Solvent	Conditions	Predicted Value	Experimental Value	Percentage Error (%)
TPC	70%	65 °C, 38 min	4.6	4.31 ± 0.12	6.30%
	Ethanol				
	70%	52 °C, 35 min	1.52	1.40 ± 0.05	7.89%
	Methanol				
TFC	70%	65 °C, 38 min	6.8	6.29 ± 0.18	7.50%
	Ethanol				
	70%	52 °C, 35 min	2.35	2.16 ± 0.09	8.09%
	Methanol				
Antioxidant activity	70%	65 °C, 38 min	85	79.8 ± 1.5	6.12%
	Ethanol				
	70%	52 °C, 35 min	74	69.7 ± 1.2	5.81%
	Methanol				

* Experimental values are presented as Mean $\pm$ SD (n = 3)

The table indicates that ethanol is a more effective solvent than methanol for extracting bioactive compounds under the studied conditions. At 65 °C for 38 minutes, ethanol produced nearly three times higher total phenolic content compared to methanol extracted at 52 °C for 35 minutes, consistent with findings that solvent polarity and hydrogen-bonding interactions influence phenolic and flavonoid solubility and extraction efficiency. Previous research has shown that aqueous ethanol often yields higher flavonoid extraction and greater antioxidant activity compared to aqueous methanol in plant materials, reflecting solvent-dependent extraction patterns (Do et al., 2014).

Consequently, the ethanol extract exhibited significantly greater antioxidant activity (85%) than the methanol extract (74%), reinforcing the positive correlation between the concentration of extracted phenolics and flavonoids and antioxidant potential observed in other plant systems. Overall, despite requiring slightly higher temperature and longer extraction time, ethanol proved superior in maximizing extraction of key antioxidant compounds and enhancing antioxidant potential, a trend similarly reported in studies where ethanol-based extracts showed stronger radical scavenging effects than methanol-based extracts in selected plant matrices (Przybylski et al., 2025).

Based on the RSM optimization using ethanol as the extraction solvent, the optimal condition for simultaneously maximizing total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA) was achieved at an extraction time of approximately 30 minutes and a temperature of around 65 °C (Figure 10). Under these conditions, the model predicted high responses with TPC of about 4.60 mg/g, TFC of approximately 6.80 mg/g, and antioxidant activity reaching 85%, indicating a strong positive influence of temperature and moderate extraction time on the release of bioactive compounds. The desirability value (~0.75) further confirms that this combination represents a suitable compromise to optimize all responses simultaneously, suggesting that higher temperatures enhance solvent penetration and mass transfer efficiency, while excessive extraction time is not required when ethanol is used as an effective and green solvent. When compared with methanol as the extraction solvent (Figure 11), ethanol demonstrated

superior performance in optimizing TPC, TFC, and antioxidant activity under the RSM-identified conditions. Although methanol also showed an increase in these responses with increasing temperature and time, the maximum predicted values obtained using methanol were consistently lower than those achieved with ethanol. Specifically, methanolic extraction generally required slightly lower optimal temperatures and shorter extraction times, yet resulted in reduced TPC and TFC yields as well as weaker antioxidant activity (74%) compared to ethanol (85%). This difference can be attributed to ethanol's better affinity for a wider range of phenolic and flavonoid compounds, as well as its enhanced ability to disrupt plant cell matrices at elevated temperatures. Overall, the comparison indicates that ethanol not only provides higher extraction efficiency for bioactive compounds from *Caesalpinia sappan* L. but also offers a more effective and safer solvent option than methanol for maximizing antioxidant-related responses.

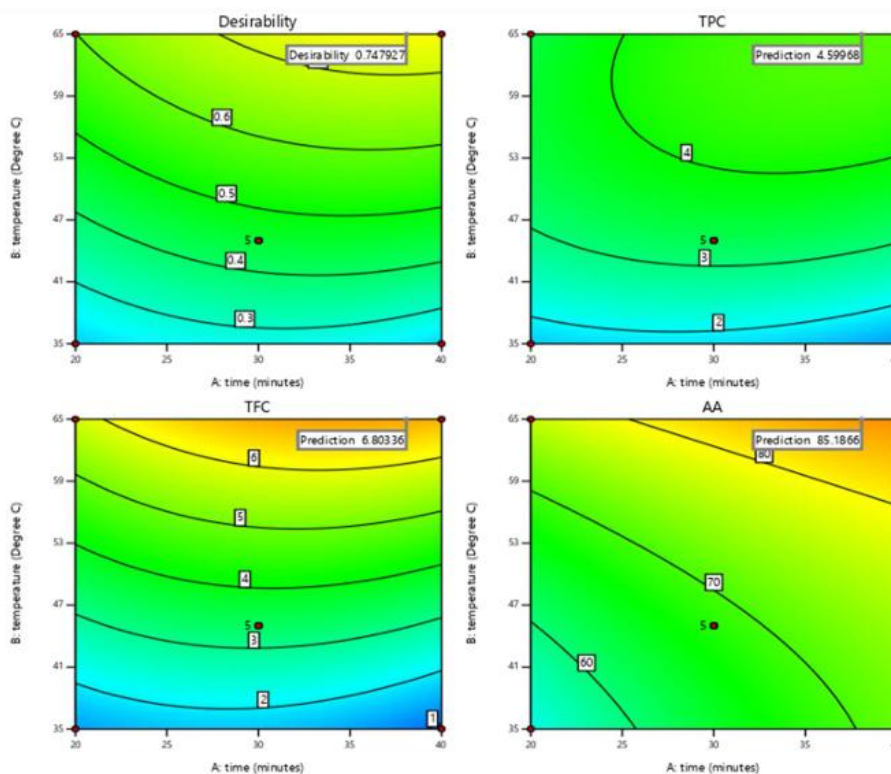


Figure 10. Graph optimization condition using ethanol as solvent

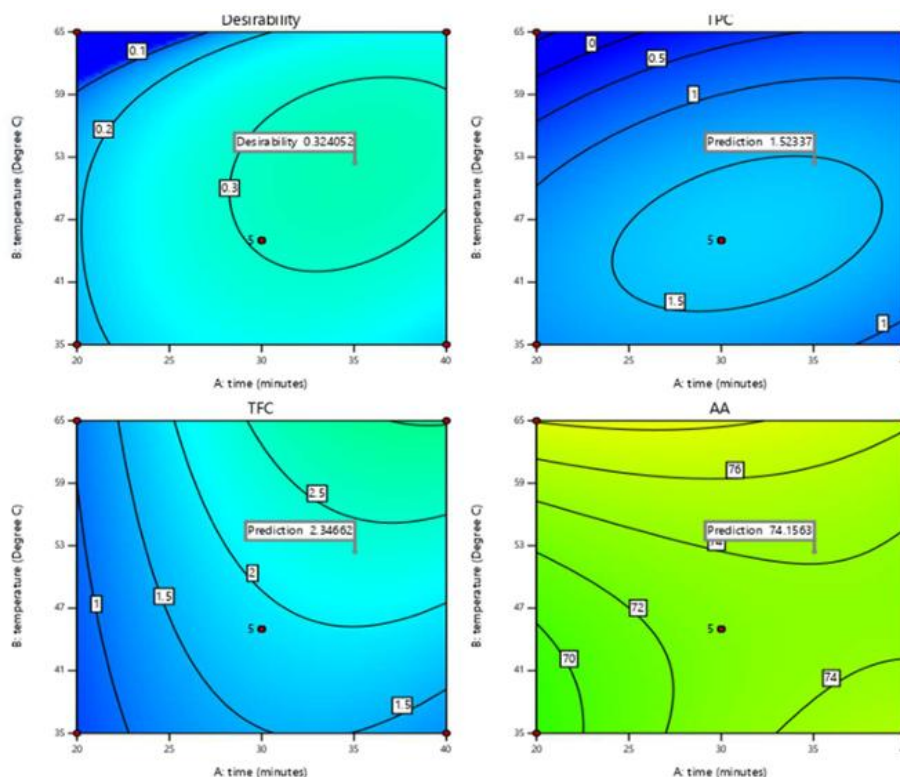


Figure 11. Graph optimization condition using methanol as solvent

Both total phenolic content, total flavonoid content, and antioxidant activity were maximized when ethanol was employed as the extraction solvent, indicating its superior extraction efficiency compared to methanol. This can be attributed to the favourable polarity of ethanol, which enables effective solubilization of a wide range of polyphenolic compounds while maintaining compound stability during extraction (Do et al., 2014). In addition to its extraction performance, ethanol is widely recognized as a food-grade solvent and is extensively used in food additives, personal care products, and alcoholic beverages, whereas methanol is primarily limited to industrial applications due to its higher toxicity (Bulk Chemicals, 2021). Considering the intended application of *Caesalpinia sappan* L. as a natural food colourant, the use of ethanol is particularly appropriate, as it ensures both high bioactive compound recovery and compliance with food safety requirements. Overall, these findings confirm that ethanol is the most suitable solvent for extracting bioactive compounds from *Caesalpinia sappan* L., supporting its potential application in food and nutraceutical industries.

4. Conclusions

This study compared 70% aqueous ethanol and 70% aqueous methanol for extraction of phenolic and flavonoid constituents and antioxidant activity from *Caesalpinia sappan* L. heartwood within 30–70 °C and 15–45 minutes. The results clearly demonstrate that ethanol is the more effective solvent for recovering phenolic and flavonoid compounds, reported experimental TPC and TFC values were 7.8 and 8.1 mg/mL, respectively, and the highest reported DPPH inhibition for ethanol was 93% at 65 °C and 40 minutes. RSM predicted solvent-specific compromise conditions of 65 °C for 38 minutes for ethanol and 52 °C for 35 minutes for methanol. This study confirms the suitability of ethanol as an efficient and safer food-grade solvent for the extraction of bioactive compounds from *Caesalpinia sappan* L., highlighting its potential application in food, pharmaceutical, and cosmetic industries.

5. References

Cacace, J. E. & Mazza G. (2003). Optimization of Extraction of Anthocyanins from Black Currants with Aqueous Ethanol. *Journal of*

- Food Science*, 68(1), 240–248.
<https://doi.org/10.1111/j.1365-2621.2003.tb14146.x>
- Che Sulaiman, I. S., Basri, M., Fard Masoumi, H. R., Chee, W. J., Ashari, S. E., & Ismail, M. (2017). Effects of temperature, time, and solvent ratio on the extraction of phenolic 37 surface compounds and the anti-radical activity of *Clinacanthus nutans* Lindau leaves by response methodology. *Chemistry* <https://doi.org/10.1186/s13065-017-0285-1>
- Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296–302.
<https://doi.org/10.1016/j.jfda.2013.11.001>
- Ghazzawi, H. A., Al-Sayyed, H. F., Al-Kurd, R. A., Mwalla, M. M., Arafat, T. A., & AbdelQader, S. M. (2021). Effect of different extraction solvents on the antioxidant content and capacity of nine seasonal fruits. *Clinical Nutrition Open Science*, 38, 3342.
<https://doi.org/10.1016/j.nutos.2021.06.003>
- Insuan, W., Sillawatthumrong, N., Chahomchuen, T., Khamchun, S., Chueahongthong, F., & Insuan, O. (2024). Brazilin content and potential biological properties of *Caesalpinia sappan* L. heartwood extracts from different extraction methods. *Discover Applied Sciences*, 6(10).
<https://doi.org/10.1007/s42452-024-06222-4>
- Karneng, S. (2024). Determination of Total Flavonoid Contents of The Antioxidant Herbal Beverage *Caesalpinia sappan* L. Quantitatively Using The Calorimetry Methods of The $AlCl_3$ Reaction That Forms Complex Compounds. *Hayyan Journal* 1(1), 57-63.
<https://doi.org/10.51574/hayyan.v1i1.1264>
- Koeswantika Putri, D., & Indah Sabila, F. (2021). Antioxidant Activity from The Combination Ethanol Extract Secang Wood (*Caesalpinia sappan* L.) And Red Ginger Rhizome (*Zingiber officinale* Roxb.) *Advances in Engineering Research*, 209.
<https://doi.org/10.2991/aer.k.211215.027>
- Kurniasari, L., Kumoro, A. C., & Djaeni, M. (2024). Ultrasound assisted extraction of sappan wood (*Caesalpinia sappan* L.) using different solvents. *Food Research*, 8, 2328.
[https://doi.org/10.26656/fr.2017.8\(S1\).4](https://doi.org/10.26656/fr.2017.8(S1).4)
- Lapornik, B. Prosek, M. & Wondra, A. G. (2005). Comparison of Extracts Prepared from Plant By-Products Using Different Solvents and Extraction Time. *Journal of Food Engineering*, 71, 214–222.
<https://doi.org/10.1016/j.jfoodeng.2004.10.036>
- Maccarronello, A. E., Cardullo, N., Margarida Silva, A., Di Francesco, A., Costa, P. C., Rodrigues, F., & Muccilli, V. (2024). From waste to bioactive compounds: A response surface methodology approach to extract antioxidants from *Pistacia vera* shells for postprandial hyperglycaemia management. <https://doi.org/10.1016/j.foodchem.2024.138504>
- Mansour, R. Ben, Falleh, H., Hammami, M., Barros, L., Petropoulos, S. A., Tarchoun, N., & Ksouri, R. (2023). The Use of Response Surface Methodology to Optimize Assisted Extraction of Bioactive Compounds from *Cucurbita maxima* Fruit By-Products. *Processes*, 11(6).
<https://doi.org/10.3390/pr11061726>
- Masturi, Alighiri, D., Edie, S. S., Hanasyifa, U., & Drastisianti, A. (2021). Determination of total phenol and flavonoid contents and antioxidant activity from extract fraction of sappan wood (*Caesalpinia sappan* l.) by liquid-liquid extraction and vacuum liquid chromatography. *Asian Journal of Chemistry*, 33(8), 1729-1735.
<https://doi.org/10.14233/ajchem.2021.23029>
- Przybylski, R., Al-Ghamdi, H. O., & Daun, J. K. (2025). Effect of the extraction solvent and method on the determination of the total polyphenol content in different common buckwheat (*Fagopyrum esculentum* Moench) varieties. *Food & Nutrition Research*, 69.
<https://doi.org/10.29219/fnr.v69.9834>

- Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., Almikhlaifi, M. A., Alghamdi, S. Q., Alruwaili, A. S., Hossain, M. S., Ahmed, M., Das, R., Emran, T. Bin, & Uddin, M. S. (2022). Role of phenolic compounds in human disease: Current knowledge and future prospects. *Molecules* 27(1), MDPI. <https://doi.org/10.3390/molecules27010233>
- Rohmah, A., Hafshah, M., & Mardliyah, A. (2022). Potential of Secang Wood (*Caesalpinia sappan* L.) Ethanol Extract as Antioxidant and Sun-Protection. *Al-Kimia* 10(2), 126–132. <https://doi.org/10.24252/al-kimia.v10i2.28619>
- Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, Isolation And Characterization Of Bioactive Compounds From Plants' Extracts. *African Journal of Traditional Complementary and Alternative Medicines*, 8(1), 1-10. <https://doi.org/10.4314/ajtcam.v8i1.60483>
- Seo, J., Lee, S., Elam, M. L., Johnson, S. A., Kang, J., & Arjmandi, B. H. (2014). Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. *Food Science & Nutrition*, 2(2), 174–180. <https://doi.org/10.1002/fsn3.91>
- Settharaksa, S., Monton, C., & Charoenchai, L. (2019). Optimization of *Caesalpinia sappan* L. heartwood extraction procedure to obtain the highest content of brazilin and greatest antibacterial activity. *Journal of Integrative Medicine*, 17(5), 351–358. <https://doi.org/10.1016/j.joim.2019.05.003>
- Taveepanich, S., Chayajarus, K., Jittimane, J., Phusri, N., Thongdee, P., Sawatdee, K., Kamsri, P., Punkvang, A., Suttisintong, K., Pungpo, P., Suwannaloet, W., Thongrungsakul, R., & Pangjit, K. (2024). Iron chelating, antioxidant, and anti-inflammatory properties of from *Caesalpinia sappan*, 10(19), e38213. <https://doi.org/10.1016/j.heliyon.2024.e38213>
- Vij, T. (2023). A narrative review on Sappan wood (*Caesalpinia sappan* L.). *Pharma Innovation*, 12(5), 2861-2865.
- Vij, T., Anil, P. P., Shams, R., Dash, K. K., Kalsi, R., Pandey, V. K., Harsányi, E., Kovács, B., & Shaikh, A. M. (2023). A Comprehensive Review on Bioactive Compounds Found in *Caesalpinia sappan*. *Molecules* 28(17). (MDPI). <https://doi.org/10.3390/molecules28176247>