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Screening of antioxidant active fraction of *Uncaria gambir* Roxb (Bajakah kalawit) wood extract and determination of total phenolics and flavonoids

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Abstract

Bajakah Kalawit wood (*Uncaria gambir* Roxb.) has strong antioxidant activity because it contains phenolic and flavonoid compounds that can capture free radicals. This study aims to find the active fraction of *Uncaria gambir* Roxb. extract as an antioxidant and determine the correlation between total phenolic content (TPC) and total flavonoid content (TFC) to antioxidant activity (IC₅₀). *Uncaria gambir* Roxb. extract was obtained using maceration method with 96% ethanol solvent then fractionated with *n*-hexane, ethyl acetate and methanol solvents. Analysis of fractions included TPC using Folin-Ciocalteu method with gallic acid standard, TFC using AlCl₃ method with quercetin standard, and antioxidant using DPPH method with quercetin comparator. The results showed that the TPC of *Uncaria gambir* Roxb. *n*-hexane, ethyl acetate and methanol solvents were 85.37±6.10; 129.30 ± 2.59; and 214.33 ± 5.77 mg GAE/g respectively. TFC of *Uncaria gambir* Roxb. Fractions of *n*-hexane, ethyl acetate and methanol were 9.08 ± 0.07; 12.40 ± 0.44; and 22.96 ± 0.80 mg QE/g respectively. The IC₅₀ of *Uncaria gambir* Roxb. fractions of *n*-hexane, ethyl acetate, and methanol solvents were 75.78±1.50; 50.27±0.71 and 46.88 ± 0.42 µg/mL. The antioxidant of *n*-hexane and ethyl acetate fraction were classified as strong antioxidant and methanol fraction was very strong antioxidant. There is a very strong positive correlation with a correlation coefficient (*r*) value of 0.954; 0.917 and 0.936 between total phenolic content (TPC) and total flavonoid content (TFC) to antioxidant activity (IC₅₀) fractions of *Uncaria gambir* Roxb.

Keywords: *Uncaria gambir*, antioxidant, total flavonoids content, total phenolic content.

Background

Degenerative diseases caused by free radicals lead to a broad spectrum of symptoms, including hypertension, diabetes, atherosclerosis, cancer, stroke, heart disease, and others (Kim et al., 2015) (Mahfudh et al., 2024). Free radicals are needed for the body's immune function, but oxidative damage at the organ level can occur if the concentration of free radicals is too

high. Free radical compounds can easily form new compounds because of their unstable and highly reactive nature, so antioxidants are needed to reduce or inhibit these new radical compounds (Valko et al., 2007).

A natural source of antioxidants is found in Bajakah kalawit wood with the scientific name *Uncaria gambir* Roxb. is a creeping shrub that grows rapidly in the Kalimantan region (Rahmaddiansyah et al., 2024). Bajakah Kalawit wood is a plant from the Rubiaceae family and the genus *Uncaria* which contains the main component of the polyphenol class in the form of flavonoids (Munggari et al., 2022). *U. gambir* has been widely used to treat diseases traditionally including diarrhea, dysentery, sore throats, and wound dressings (Hasna et al., 2021). Besides, *U. gambir* is also used as a mouthwash as well as astringent drugs for cosmetics or beauty treatments (Amir et al., 2012). In addition, the Dayak tribe often utilizes Bajakah Kalawit for the treatment of cancer and diabetes mellitus (Zein et al., 2022).

In the health sector, flavonoids have pharmacological activities as antidiabetics, antibacterials, anticancer, and antioxidants. Strong antioxidant activity is found in Bajakah Kalawit wood because it contains many compounds that can reduce free radicals, namely phenolic compounds and flavonoids. The glucoside form of flavonoids functions as an antioxidant with its mechanism of donating hydrogen atoms to radical compounds to produce more stable nonradical compounds (Aprely et al., 2021).

Previous research reported antioxidant activity of Bajakah Kalawit wood methanol extract based on IC₅₀ value of 18.27 µg/mL, while total phenolic content and total flavonoids content were 18.37 mg GAE/g dry weight and 5.82 mg RE/g dry weight, respectively (Amir et al., 2012). The antioxidant activity of *gambir* extract using ethyl acetate solvent amounted to 25.55 µg/mL, while phytochemical screening showed that *gambir* extract contained flavonoids, phenolics, and saponins (Melia et al., 2015). In another study, the IC₅₀ value of *gambir* stem extract using *n*-hexane solvent was 107.11 µg/mL; ethyl acetate 23.39 µg/mL; and methanol 9.71 µg/mL (Hidayati & Rahmatulloh, 2022).

Several studies have reported on the content of secondary metabolites in Bajakah Kalawit wood, while reports on the correlation between total phenolic content (TPC) and total flavonoid content (TFC) to antioxidant activity are still limited. Therefore, it is necessary to search for active fractions of Bajakah Kalawit wood (*Uncaria gambir* Roxb.) extract and determine the correlation between TPC and TFC to antioxidant activity of Bajakah Kalawit wood fractions with different levels of polarity, namely *n*-hexane, ethyl acetate and methanol. Determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method with Inhibitor Concentration (IC₅₀) parameter.

Screening of the active fraction of Bajakah Kalawit wood extract to determine the distiller that is able to produce the maximum antioxidant effect characterized by a smaller IC₅₀ value, which contains the most secondary metabolites in the form of phenolic compounds and flavonoids. So that the results of this study can be the basis for the development of traditional medicine Bajakah Kalawit wood as an antioxidant.

1. Materials and Methods

The materials used were Bajakah Kalawit (*Uncaria gambir* Roxb.) wood from South Kalimantan and authenticated in Laboratory of Biology Universitas Lambung Mangkurat.

1.1. Extraction

Bajakah Kalawit wood is sieved using a 40 mesh sieve to obtain rough powder of bajakah kalawit wood. Then the crude powder was weighed as much as 200 g and extracted using the maceration method for 3 days with 96% ethanol solvent as much as 2L. This process was repeated by remaceration until the solvent became clear. The resulting marinade was then filtered using a buchner funnel and concentrated using a rotary evaporator at a temperature of 60°C and 100 rpm speed. Furthermore, the yield calculation was carried out to determine the extraction results obtained.

1.2. Fractionation

The chart for the preparation of Bajakah Kalawit wood fractions can be seen in Figure 1.

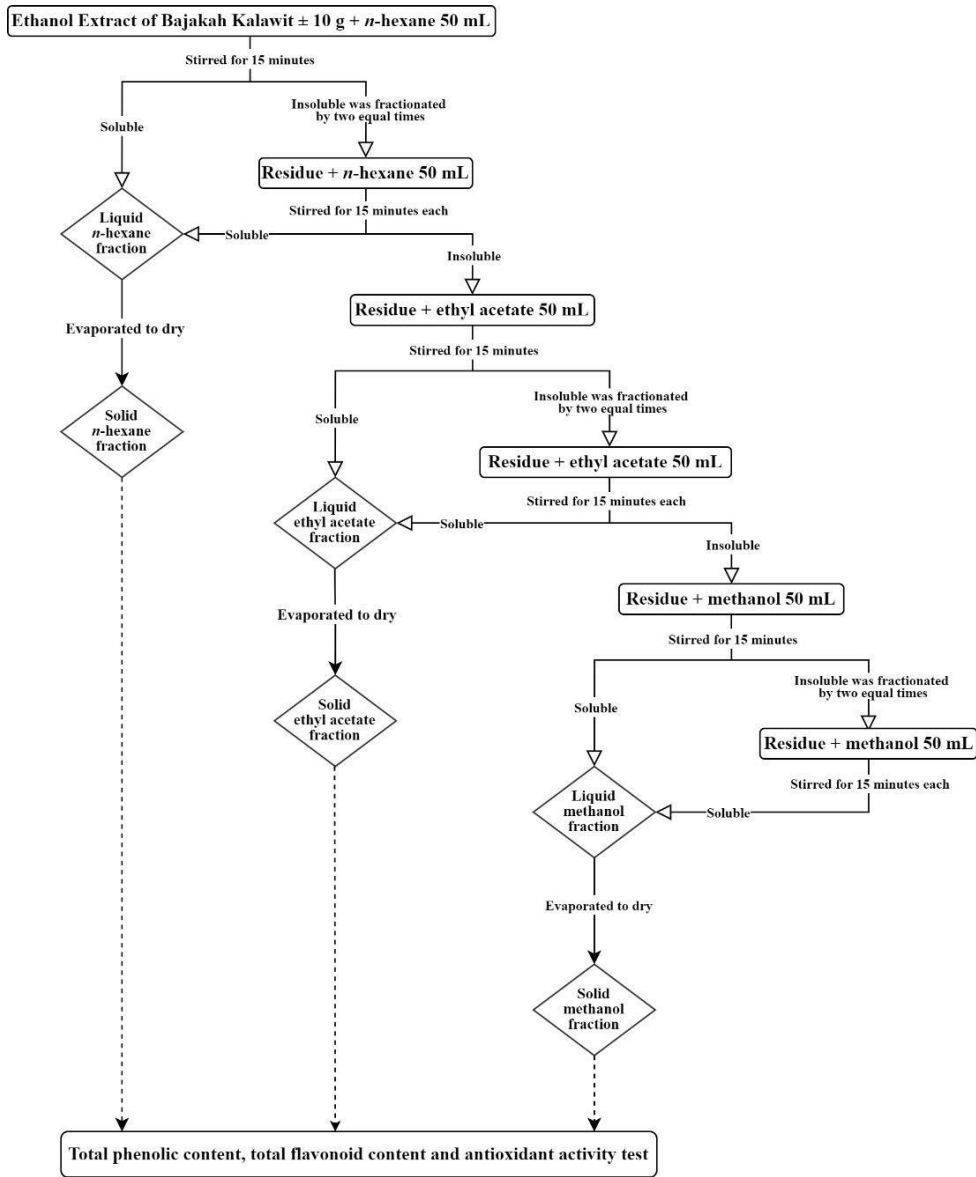


Figure 1. Fractionation of Bajakah Kalawit Wood.

2.2. Total Phenol Content Analysis (TPC)

Each fraction sample weighed carefully ± 0.2 g was diluted with 25.0 mL methanol P solvent. In each fraction, 1.0 mL was taken and 5.0 mL of Folin Ciocalteu LP reagent was added and allowed to stand for 8 minutes. After that, 4.0 mL of 1% NaOH was added to the reaction mixture and then shaken and incubated for 1 hour. Absorbance measurements were

taken on a UV-Vis spectrophotometer at a wavelength 730 nm. Preparation of standard curve using gallic acid standard (Anesini et al., 2008).

2.3. Total Flavonoid Phenol Content Analysis (TPC)

Each fraction sample was carefully weighed ± 0.2 g diluted with 25.0 mL methanol solvent. Then each fraction was taken as much as 0.5 mL and added ethanol P 1.5 mL; AlCl_3 P 10% 0.1 mL; CH_3COONa 1M 0.1 mL; and water 2.8 mL. After that, the reaction mixture was allowed to stand for 30 minutes. Absorbance measurements were performed on a UV-Vis spectrophotometer with a wavelength of 510 nm (Chang et al., 2002). Preparation of standard curve using quercetin as standard.

2.4. Antioxidant Activity Analysis

Quercetin standard was carefully weighed ± 10.0 mg and then dissolved with 100.0 mL ethanol P and obtained a concentration of 100 ppm. Then a series solution of 2; 4; 6; 8; and 10 ppm was made. Each fraction of Bajakah Kalawit wood was carefully weighed ± 80.0 mg and dissolved with ethanol 100 mL. Furthermore, each fraction solution was made five series of levels of 20; 40; 60; 80; and 100 ppm. Each fraction sample solution and quercetin comparator was taken 1.0 mL and dissolved with 1.0 mL of 0.15 mM DPPH solution and then allowed to stand for 1 hour in a place protected from light. Absorbance measurements were taken on a UV-Vis spectrophotometer with a wavelength of 517 nm. Replication was done five times (Nurhasnawati et al., 2019).

2.5. Statistical Analysis

The data obtained were then statistically tested by utilizing statistical software, namely SPSS version 26.0 with a confidence level of 95%. Data were analyzed in three stages, namely post-hoc anova analysis, correlation and multiple linear regression.

2. Results and Discussion

3.1 Fractionation, Total Phenolic Content (TPC), and Total flavonoid content (TPC)

Uncaria gambir is a potential medicinal plant has been used for treatment of various diseases traditionally. The identification of active compound is an important stage of development of medicinal plant. The fractionation process aims to separate secondary metabolite compounds contained in ethanol extracts based on their polarity. Based on the

polarity index, *n*-hexane solvent has a value of (0.1), ethyl acetate (4.4) and methanol (5.1). The greater the polarity index value, the more polar it will be. The yield of extracts and fractions of Bajakah Kalawit wood and the TPC and TFC were presented in Table 1.

Table 1. Yield, TPC, and TFC on Each Bajakah Fractions

Fractions	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
<i>n</i> -Hexane Fraction	18.32	85.37 ± 6.10	9.08 ± 0.07
Ethyl Acetate Fraction	31.24	129.30 ± 2.59	12.40 ± 0.44
Methanol Fraction	21.52	214.33 ± 5.77	22.96 ± 0.80

Furthermore, determination of total phenolic content was carried out using the Folin-ciocalteu method. The reaction is based on the reducing ability of the phenolic group to heteropoly acid or phosphomolybdate phosphotungstat contained in Folin-ciocalteu and reacts into a concentrated blue molybdenum-tungsten complex compound. The results of the TPC test for Bajakah Kalawit wood fractions can be seen in Table 1.

Based on the data in Table 1, it is known that the average levels of total phenolics in the *n*-hexane, ethyl acetate and methanol fractions are 85.37 ± 6.11 mg GAE/g; 129.30 ± 2.59 mg GAE/g and 214.33 ± 5.77 mg GAE/g, respectively. Phenolic compounds have a greater content in polar fractions, indicating that the phenolic content in *Uncaria gambir* is polar. The previous research reported total phenolic content of the methanol extract of *Uncaria gambir* leaves amounted to 18.37 mg GAE / g ± 2.79 (Amir et al., 2012) and on ethyl acetate extract of *U. gambir* leaves powder amounted to 272.875 mg GAE/g (Melia et al., 2015).

Determination of total flavonoid content was carried out using the AlCl₃ method. The reaction is based on the ability of AlCl₃ to oxidize the flavonoid group, namely the keto-hydroxyl and reacts into a stable complex compound with a concentrated yellow color (Arifin et al., 2021). The results of the TFC test of Bajakah Kalawit wood fractions can be seen in Table 1. Based on the data in Table 1, it is known that the average levels of total flavonoids in *n*-hexane, ethyl acetate and methanol fractions are 9.08 ± 0.07 mg QE/g; 12.40 ± 0.44 mg QE/g and 22.96 ± 0.80 mg QE/g, respectively. The previous study reported the TFC of the methanol extract of *U. gambir* leaves amounted to 5.82 ± 2.23 mg RE/g (rutin equivalent per gram extract). While the fraction of *U. gambir* leaves in methanol solvent amounted to 11.52 mg RE/g ± 0.02) and ethyl acetate solvent of 2.90 mg QE/g ± 0.04 (Pambayun et al., 2007).

3.2 Inhibition Activity of DPPH radicals

Quantitatively, the ability of the sample to reduce DPPH radicals is indicated by the decrease in absorbance of the test solution calculated against the control solution. The smaller the absorbance value, the stronger the ability of the test sample to reduce DPPH radicals or the stronger the antioxidant. The data obtained from the measurements are % inhibition and concentration of the test compound then processed using linear regression analysis to obtain the concentration of radical capture as much as 50% (IC_{50}). The correlation between the extract concentration and the capacity of inhibition of DPPH was presented in Figure 1. The results of antioxidant activity test, which stated as IC_{50} values can be seen in Figure 2.

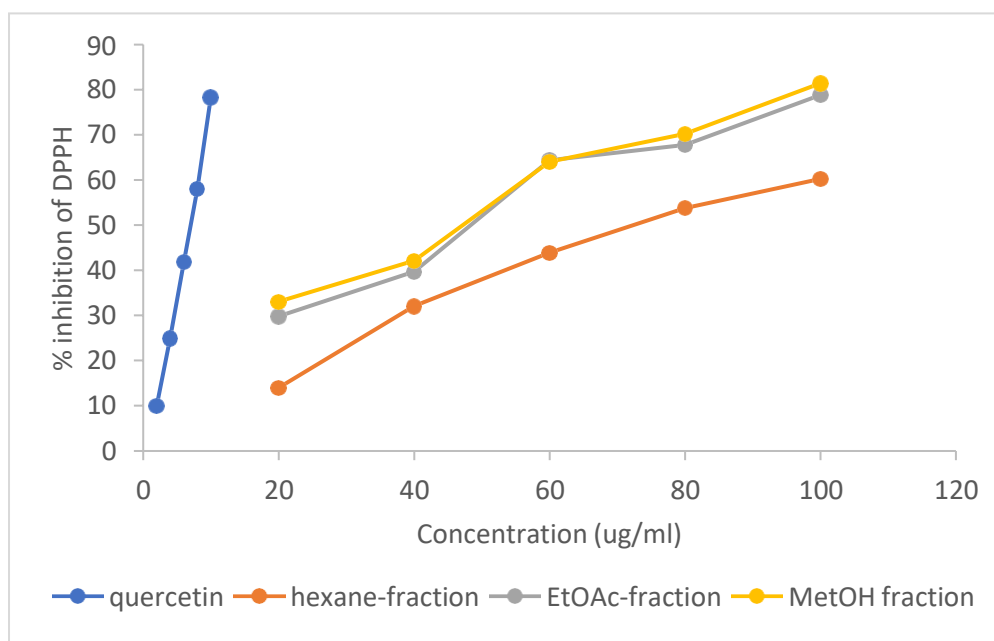


Figure 1. The correlation between concentration and capacity of inhibition of

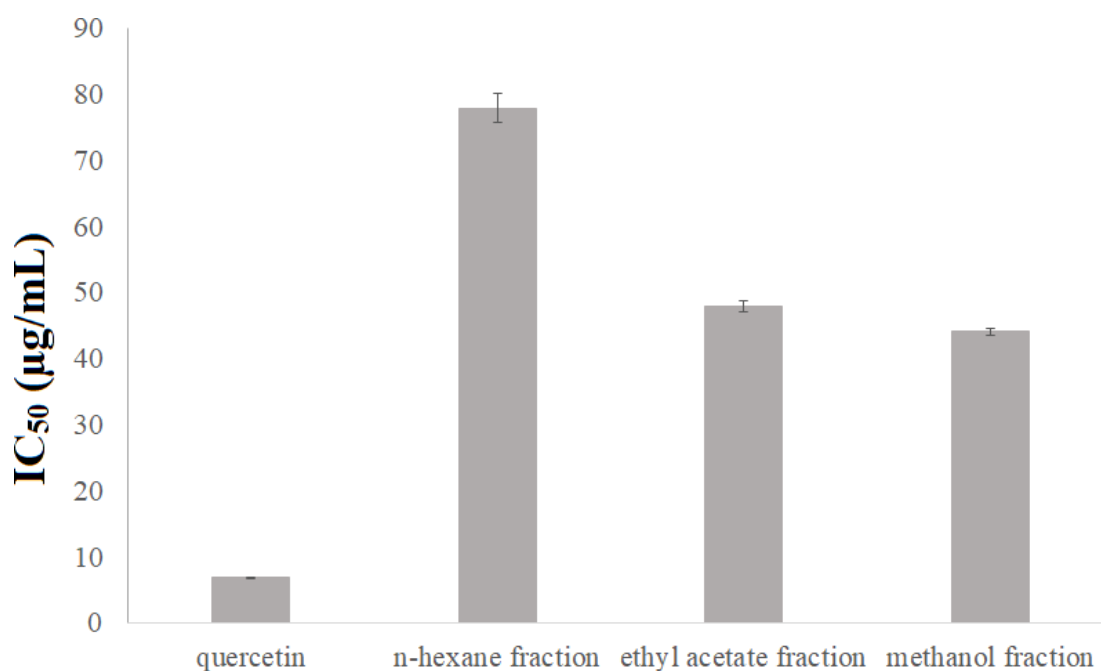


Figure 2. The IC₅₀ value from various Bajakah fractions

The average value of IC₅₀ of quercetin standard is 6.87 ± 0.08 µg/mL which is classified as a very strong antioxidant because the value of IC₅₀ < 50 µg/mL (Molyneux, 2004). Quercetin is classified as a very strong antioxidant and the calculation results show the higher the concentration, the higher the % inhibition of DPPH radicals. So it can be concluded that the greater the concentration of quercetin standard, the antioxidant activity also increases. The hydroxyl group in the quercetin molecule could have important role in its antioxidant activity (Alrawaiq & Abdullah, 2014).

The average IC₅₀ values of *n*-hexane, ethyl acetate, and methanol fractions are 75.78 ± 1.50 µg/mL, 50.27 ± 0.71 µg/mL, and 46.88 ± 0.42 µg/mL are classified as very strong antioxidants. The strong antioxidant activity of *U. gambir* extract was also showed by FRAP method, with an IC₅₀ value of ethanol extract, water fraction and *n*-hexane fraction were 29.98 µg/mL, 39.8 µg/mL, and 75.32 µg/mL, respectively (Siti Nur Indriyah et al., 2023). Other studies also reported that the polar fractions (methanol and ethyl acetate) of *U. gambir* have a better antioxidant activity compare to non-polar fraction (*n*-hexane) (Hidayati & Rahmatulloh, 2022; Melia et al., 2015). Based on the results of these studies, it can be concluded that the antioxidant activity of Bajakah Kalawit wood fraction which showed by IC₅₀ values from the highest to the lowest value is methanol, ethyl acetate, and *n*-hexane, respectively.

The most active fraction of Bajakah Kalawit wood extract that has the highest antioxidant activity is the methanol fraction with an IC_{50} value of $46.88 \mu\text{g/mL} \pm 0.42$ with a very strong antioxidant category. Methanol is a solvent at the third level used to make fractions with the most polar nature among solvents in other samples, namely *n*-hexane and ethyl acetate. The results of antioxidant activity data obtained in each sample are also influenced by the content of chemical compounds in it in the form of phenolic compounds and flavonoids that can dissolve in polar solvents.

Based on Table 1, the yield value is related to how much chemical compound content in the fraction sample. If the resulting yield value is greater, the content of chemical compounds in the sample of interest is more and more. The results of the calculation of the yield of this study in the *n*-hexane, ethyl acetate and methanol fractions were 18.32%; 31.24% and 21.52%, respectively. The highest value of yield calculation results was obtained by the ethyl acetate fraction, but in the calculation of TPC, TFC and IC_{50} the highest value was obtained by the methanol fraction. This can be caused by contamination by foreign particles during the fractionation process so that it can reduce the yield value, besides that it can be caused by accidental leakage or spillage in the container. It is known that phenolics and flavonoids exhibit antioxidant activity and their effect on nutrition and human health is considerable. Correlation analysis on several Zingiberaceae plants showed that TPC and TFC were significant contributors to antioxidant activity (Fitriansyah et al., 2017; Maulana et al., 2019). In addition, some Umbelliferae plants show a strong positive correlation that TPC and TFC are the main substances that contribute significant amounts to antioxidant activity (Kim & Lee, 2020).

3.2. Correlation of phenolic and flavonoid content to antioxidant activity

The TPC, TFC, and IC_{50} data obtained in each fraction were statistically analyzed with a confidence level of 95% using SPSS (Statistical Product and Service Solution) statistical software version 26.0. The statistical analysis method used is Spearman Correlation as listed in Table 2 and Figure 3. Based on the results of statistical analysis, it shows that there is a correlation or correlation between TPC to TFC, TPC to antioxidant activity and TPC to antioxidant activity because the Sig. (2-tailed) value $<0,05$. The correlation coefficient values shows that there is a very strong positive correlation or in other words, the increasing of TPC and TFC, the antioxidant activity increases as well.

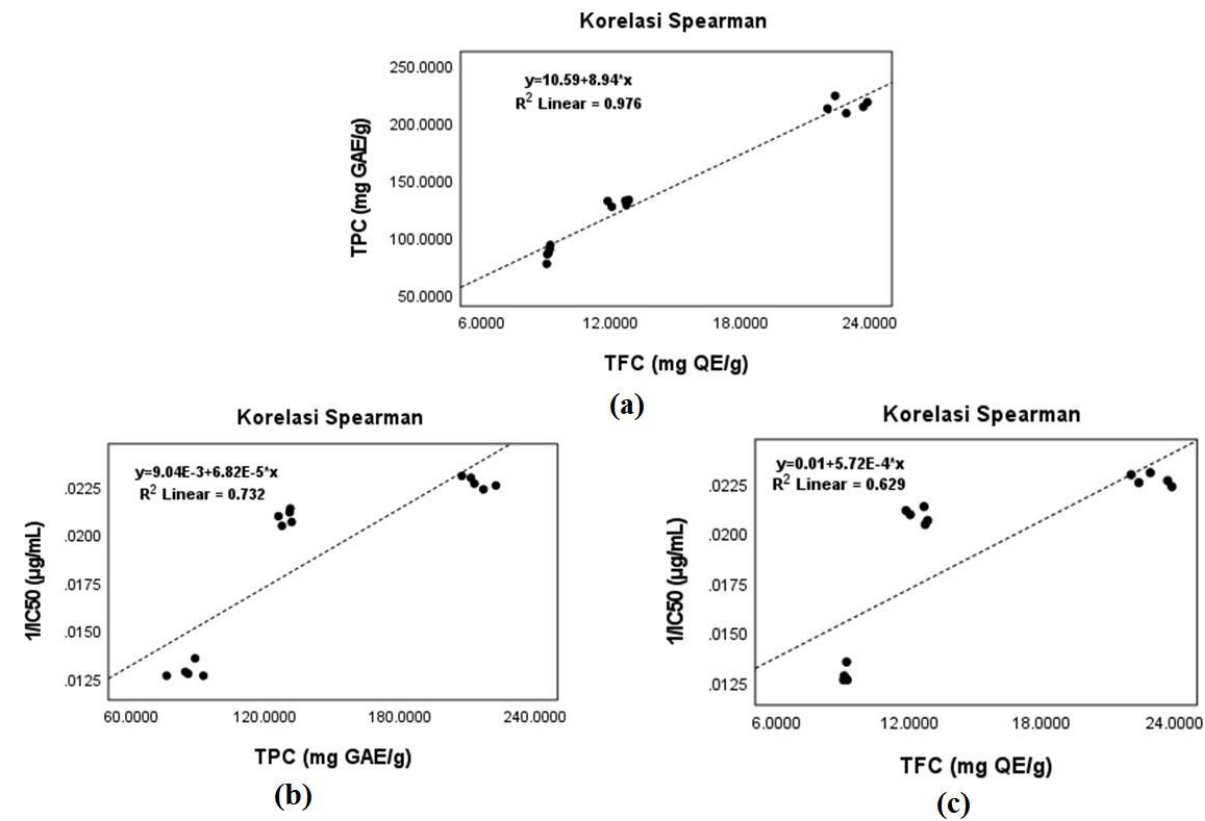


Figure 3. The correlation between: a) TFC vs TFC; b) 1/IC₅₀ vs TPC; c) 1/IC₅₀ vs TFC

Table 2. Spearman Correlation Analysis Results.

Variables	TPC	1/IC ₅₀ µg/mL
TPC	1	0.872*
TFC	0.954*	0.858*
1/IC ₅₀ µg/mL	0.872*	1

IC₅₀: inhibition concentration of 50%; *calculated using spearman method (p<0.05 has a good correlation)

3. Conclusion

The antioxidant activity (IC₅₀) of Bajakah Kalawit Wood Fractions in n-hexane, ethyl acetate and methanol solvent were 75.78 ± 1.50 µg/mL classified as strong; 50.27 ± 0.71 µg/mL classified as strong and 46.88 ± 0,42 µg/mL classified as very strong, respectively. The most active fraction of Bajakah Kalawit wood is the methanol fraction. There is a very strong positive correlation between TPC, TFC and antioxidant activity (IC₅₀) of Bajakah Kalawit wood.

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