Jurnal Pharmascience, Volume 13, Issue 1, March 2026, page: 236-254
ISSN-Print 2355 – 5386
ISSN-Online 2460-9560
https://dx.doi.org/10.20527/jps.v13i1.24662
Research Article

Evaluation of Extraction Methods on the Antioxidant Potential of Balik Angin Leaves Ethanol Extract (*Alphitonia incana* (Roxb.) Teijsm. & Binn. ex Kurz)

Evaluasi Metode Ekstraksi terhadap Potensi Antioksidan Ekstrak Etanol Daun Balik Angin (Alphitonia incana (Roxb.) Teijsm. & Binn. ex Kurz)

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ABSTRACT

***Alphitonia incana*, also known locally as balik angin, is an endemic natural antioxidant plant of Borneo. The objective of the present work was to compare the effectiveness of maceration and Soxhlet extraction of balik angin leaves with respect to antioxidant activity and phenolic–flavonoid content. Ethanolic extracts were prepared using both extraction methods, and the phenolic-flavonoid content was quantified spectrophotometrically. Antioxidant performance was assessed through DPPH, CUPRAC, and FRAP assays. The findings revealed that the Soxhlet-derived extract contained higher concentrations of phenolic-flavonoid compounds than those produced by maceration. In addition, the Soxhlet-derived extract demonstrated stronger antioxidant activity, as evidenced by lower IC_{50} and EC_{50} values in the DPPH (10.872 ppm) and CUPRAC (6.578 ppm) assays compared with the macerated extract (IC_{50} 13.127 ppm; EC_{50} 14.034 ppm) and was classified as a very strong antioxidant. The FRAP assay further supported these results, with ferric reducing power values of 1,484.1 mg AAE/g extract for the Soxhlet-derived extract and 1,295.8 mg AAE/g extract for the macerated sample. Overall, the study concludes that Soxhlet extraction is more effective than maceration in enhancing the antioxidant potential of balik angin leaf, a result that is closely associated with their elevated phenolic and flavonoid content.**

Keywords: Balik Angin Leaf, Natural Antioxidant, Maceration, Soxhlet, Endemic

ABSTRAK

Alphitonia incana, yang juga dikenal secara lokal sebagai balik angin, adalah tanaman antioksidan alami endemik Borneo. Penelitian ini bertujuan untuk membandingkan maserasi dan ekstraksi Soxhlet daun balik angin dalam menghasilkan aktivitas antioksidan dan kandungan total fenolik-flavonoid. Ekstrak etanol disiapkan menggunakan kedua metode ekstraksi, dan kandungan fenolik-flavonoid diukur secara spektrofotometri. Kinerja antioksidan diukur melalui uji DPPH, CUPRAC, dan FRAP. Hasil penelitian menunjukkan bahwa ekstrak yang dihasilkan dengan Soxhlet mengandung konsentrasi senyawa fenolik-flavonoid yang lebih tinggi daripada yang dihasilkan dengan maserasi. Selain itu, ekstrak yang dihasilkan dengan Soxhlet menunjukkan aktivitas antioksidan yang lebih kuat, sebagaimana dibuktikan oleh nilai IC_{50} dan EC_{50} yang lebih rendah dalam uji DPPH (10,872 ppm) dan CUPRAC (6,578 ppm) dibandingkan dengan ekstrak maserasi (IC_{50} 13,127 ppm; EC_{50} 14,034 ppm) dan diklasifikasikan sebagai antioksidan yang sangat kuat. Pengujian FRAP lebih lanjut mendukung hasil ini, dengan nilai daya reduksi ferri sebesar 1.484,1 mg AAE/g ekstrak untuk ekstrak yang diperoleh dengan Soxhlet dan 1.295,8 mg AAE/g ekstrak untuk sampel maserasi. Secara keseluruhan, penelitian ini menyimpulkan bahwa ekstraksi Soxhlet lebih efektif daripada maserasi dalam meningkatkan potensi antioksidan daun balik angin, hasil tersebut berhubungan erat dengan kandungan fenolik dan flavonoidnya yang tinggi.

Kata Kunci: Daun Balik Angin, Antioksidan alami, Maserasi, Soxhlet, Endemik

I. INTRODUCTION

Nowadays, the global population is experiencing a rising prevalence of degenerative diseases, including coronary heart disease, hypertension, stroke, and cancer. This increasing trend is closely associated with environmental degradation, air pollution, excessive consumption of fatty and fast foods, elevated cholesterol levels, and unhealthy habits such as smoking. These conditions promote the excessive formation of free radicals within the body. Free radicals attack unsaturated lipid compounds that have electron-rich groups. These free radicals combine with lipids to produce lipid peroxides. The most effective way to neutralize free radicals is through antioxidant activity (Gulcin, 2025; Münzel et al., 2022). Antioxidants are bioactive compounds that mitigate oxidative stress by stabilizing reactive free radicals. They function by donating electrons to these unstable species, thereby neutralizing their reactivity and preventing the initiation of harmful oxidative chain reactions. Through this mechanism, antioxidants help protect cellular components and maintain normal metabolic processes (Tumilaar et al., 2023).

Natural antioxidants are in demand by the public because natural antioxidants are safer than synthetic antioxidants.

Synthetic antioxidants have carcinogenic properties in the long-term use; thus, natural antioxidants could be an alternative treatment (Ki et al., 2024). The plant that has antioxidant properties is *Alphitonia incana* (Roxb.) Teijsm. & Binn. ex Kurz. It is locally known as balik angin, one of the endemic plants that can grow on mountain slopes on the island of Borneo, and contains flavonoids of the hydroxyauron group, which is alphitonin (Forestryana et al., 2022; Ramadhan et al., 2025). Ethnobotanical evidence indicates that balik angin has long been utilized by the Dayak community in traditional healing practices. The leaves are commonly incorporated into daily bathing routines as a natural cleansing agent for skin maintenance and are also traditionally applied in the management of various skin-related ailments (Ramadhan et al., 2023; Wardah & Sundari, 2019).

A factor influencing extract quality is the extraction method used. Two widely applied extraction methods for obtaining phytochemicals are maceration and Soxhlet extraction, each offering distinct advantages (Hidayati, 2025). As a cold extraction approach, maceration helps preserve heat-labile compounds while requiring minimal instrumentation and lower solvent consumption (Sayakti et al., 2022). The novelty of this study lies in addressing the limited quantitative and mechanistic understanding of how extraction techniques influence the antioxidant potential of *Alphitonia incana*. Although previous studies have reported antioxidant activity of methanolic leaf extracts prepared by maceration and Soxhlet extraction, yielding IC_{50} values of 13.704 ppm and 9.984 ppm, respectively, these investigations were confined to a single solvent system and primarily relied on the DPPH assay (Ramadhan et al., 2025).

Moreover, ethanol Soxhlet extracts of *A. incana* leaves have been shown to contain various secondary metabolites, including phenols, flavonoids, tannins, alkaloids, and saponins; however, their antioxidant capacity has not been quantitatively evaluated (Ramadhan et al., 2025). Earlier work on the bark extract using 70% ethanol confirmed antioxidant activity only at a qualitative level (Sutomo et al., 2016). Consequently, a significant scientific gap remains regarding the comparative efficiency of ethanol-based maceration and Soxhlet extraction in modulating antioxidant performance and phytochemical yield in *A. incana* leaves.

The present study advances current knowledge by systematically comparing these two extraction approaches using the same ethanol solvent system and evaluating antioxidant capacity through multiple complementary assays (DPPH, CUPRAC,

and FRAP), alongside quantification of total phenolic and total flavonoid contents. This integrated design provides new quantitative insights into the relationship between extraction method, phytochemical composition, and antioxidant mechanism (radical scavenging versus reducing power). By correlating extraction technique with both bioactivity and phytochemical parameters, this study offers a more comprehensive mechanistic and comparative framework than previous reports, thereby clarifying the optimal extraction strategy for maximizing the antioxidant potential of *A. incana* leaves.

II. METHOD

A. Chemicals and Instruments

Analytical-grade reagents and solvents were sourced from Merck KGaA, except for the standards quercetin, gallic acid, DPPH, and ascorbic acid, which were acquired from Sigma-Aldrich. Absorbance was measured using a PG Instruments T60 UV–Vis spectrophotometer and matched quartz cuvettes.

B. Sample Preparation

Balik angin foliage in fresh condition was obtained from Mount Tahura area, Banjar Regency, South Kalimantan. The leaves were rinsed thoroughly under running water, dried in the air away from direct sunlight, pulverized into a fine powder, and passed through a 40-mesh sieve (Cock, 2020; Fuentes et al., 2020).

C. Maceration Extraction

Fifty grams of powdered leaves were immersed in 500 mL of ethanol and left to macerate for 24 hours at ambient temperature with intermittent stirring. The extract was then filtered, and the remaining residue was subjected to two additional extraction cycles. The combined filtrates were evaporated with a rotary evaporator and water bath maintained at 50 °C until reaching a stable weight (Ramadhan et al., 2024).

D. Soxhlet Extraction

Thirty-five grams of leaf powder were wrapped in filter paper and processed by Soxhlet extraction using 250 mL of ethanol at temperatures not exceeding 60 °C until the solvent was colorless. After evaporation of the solvent at 50 °C, the extract was dried and preserved at 4 °C under light-protected conditions (Ramadhan et al., 2024).

E. Phytochemical Screening

Qualitative testing of secondary metabolites in the 70% ethanol extract of balik angin leaves involved several tests as reported by Ramadhan et al. (2020).

F. Determination of total phenolic contents (TPCs)

The total phenolic concentration of the extracts was quantified using a colorimetric approach based on the Folin–Ciocalteu reaction, with gallic acid employed as the calibration standard. The procedure involved reacting 0.5 mL of each extract (100 ppm) or gallic acid standard (35–55 ppm) with 5 mL of Folin–Ciocalteu reagent diluted 1:10. After 5 minutes, 4 mL of 1 M sodium carbonate was added, followed by dark incubation for 55 minutes before measuring absorbance at 755 nm. Phenolic content was determined and expressed as µg GAE/mg extract (Yuliani et al., 2022).

G. Determination of total flavonoid contents (TFCs)

Total flavonoid contents were measured using an $AlCl_3$-based colorimetric assay, with quercetin employed as the reference standard. Ethanolic solutions of the extracts (2000 ppm) and quercetin standards at concentrations of 10–50 ppm were prepared prior to analysis. Each sample solution (1 mL) was combined with 1 mL of 10% $AlCl_3 \cdot 6H_2O$ and subsequently diluted with 8 mL of 5% glacial acetic acid. All measurements for both extracts were conducted in triplicate. After a 20-minute incubation period, absorbance readings were recorded at 420 nm. Flavonoid content was quantified and reported as µg of quercetin equivalents per mg of extract. (Yuliani et al., 2022).

H. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

Quantitative antioxidant evaluation was conducted by combining 4 mL of each serially diluted fraction with 1 mL of a 0.4 mM DPPH solution, followed by a 30-minute incubation period to allow the reaction to proceed. The blank solution consisted of 4 mL methanol (MeOH) mixed with 1 mL of DPPH reagent. Absorbance measurements were carried out at 515 nm using a UV–Visible spectrophotometer. The identical protocol was followed for the quercetin standard. Each sample, including controls, was tested in triplicate, and DPPH radical scavenging percentages were used to calculate the IC_{50}. Inhibition (%) was obtained using the formula: [(A_control − A_sample) / A_control] × 100%. The correlation between sample concentration and scavenging activity was evaluated using a linear regression model expressed as $y = bx + a$. The IC_{50} value was subsequently derived by substituting $y = 50$ into the regression equation and calculating the corresponding x value (Purnama et al., 2022; Ramadhan & Forestryana, 2021).

I. Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

The antioxidant capacity of balik angin leaf extracts was assessed using quercetin as the reference compound. Aliquots of 1 mL from each extract were prepared at graded concentrations of 3, 6, 9, 12, and 15 ppm, while quercetin solutions were prepared in the range of 1–5 ppm, and each solution was transferred into separate vials. Subsequently, 1 mL of 0.01 M $CuCl_2 \cdot 2H_2O$, 1 mL of 0.0075 M neocuproine dissolved in ethanol, 1 mL of 1 M ammonium acetate (NH_4Ac) buffer, and 0.1 mL of distilled water were added to each vial. The reaction mixtures were incubated in the absence of light for 30 minutes. Absorbance values were recorded at 450 nm, the established maximum wavelength, with a UV–Vis spectrophotometer. Each measurement was performed in triplicate. After obtaining the absorbance values, the effective concentration required to achieve 50% activity (EC_{50}) was determined. Antioxidant capacity, expressed as a percentage, was computed using the equation: (1 − antilog absorbance) × 100%. A calibration curve plotting percentage capacity against concentration was constructed, and the EC_{50} value was calculated using the linear regression equation y = bx + a by substituting y = 50 to obtain the corresponding concentration value (Ramadhan et al., 2022).

J. Ferric Reducing Antioxidant Power (FRAP) Assay

An ascorbic acid calibration plot was established using standard solutions prepared within the 60–100 ppm range, and their absorbance values were recorded. For each standard, 1 mL of ascorbic acid solution was reacted with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$, then incubated at 50 °C for 20 minutes. Afterward, 1 mL of TCA was added, the mixture was homogenized, and centrifuged at 3000 rpm for 10 minutes. One milliliter of the supernatant was mixed with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, followed by a 10-minute incubation. Absorbance was measured at 725 nm using a UV–Vis spectrophotometer. Balik angin leaf extract solutions (100 ppm) were analyzed under identical experimental conditions. Antioxidant activity was determined using the linear regression equation (y = bx + a) from the ascorbic acid standard curve, with extract concentration obtained from the x-axis values. The activity was presented as mg AAE per g of extract (Ramadhan et al., 2024).

K. Data Analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation. Statistical analysis to evaluate differences in antioxidant activity was conducted using IBM SPSS Statistics with a significance level set at 0.05 (5%). Data normality was assessed using the Shapiro-Wilk test and homogeneity using Levene's test. When the assumptions were satisfied, the data were further analyzed using one-way ANOVA. If the assumptions of normality and homogeneity were not met, the data were analyzed using the Kruskal–Wallis test instead.

III. RESULTS AND DISCUSSION

Balik angin leaves were extracted using two techniques, namely maceration and Soxhlet extraction. The choice of extraction technique plays an important role in determining extraction efficiency, as it affects the concentration and potential therapeutic activity of plant constituents. Certain medicinal plants contain compounds that are sensitive to heat and may degrade at elevated temperatures. Therefore, both maceration and Soxhlet extraction were selected in this study, as they are widely applied extraction approaches. Differences in extraction temperature between these methods may influence the solubility of flavonoid and phenolic compounds present in balik angin leaves. Higher temperatures tend to enhance phenolic solubility in solvents by accelerating diffusion processes (Hasnaeni et al., 2019; Ramadhan et al., 2024).

Maceration and Soxhlet techniques were selected due to their distinct advantages compared with other extraction approaches. Maceration, as a low-temperature process, minimizes the risk of compound degradation and enables the recovery of a wide range of phytochemicals. In contrast, Soxhlet extraction employs heat and is considered highly effective for maximizing extract yield while requiring a smaller volume of solvent; continuous recycling of the solvent ensures thorough extraction of the sample (Cao et al., 2025). The resulting extracts were subsequently used for phytochemical screening to identify various secondary metabolites, including phenolic compounds, flavonoids, alkaloids, tannins, saponins, steroids, and triterpenoids. The outcomes of these analyses are presented in Table I.

This investigation revealed that the ethanol extract of balik angin leaves comprises multiple classes of secondary metabolites, including phenolic compounds, flavonoids, tannins, saponins, and triterpenoids. These observations are consistent with previous work by Ramadhan et al. (2023), who reported that

ethanol-based maceration of balik angin leaves effectively isolates phenolic and flavonoid constituents from the plant material. Polyphenols represent a class of bioactive, non-nutritional plant metabolites that have been widely explored for their role in the prevention and management of disorders associated with oxidative stress (Kapoor et al., 2025). Among phenolic compounds, flavonoids constitute the largest and most prevalent subgroup and are recognized for their function as naturally occurring antioxidants (Tungmunnithum et al., 2018). Numerous biochemical activities have been attributed to flavonoids; however, their antioxidant capacity remains the most consistently documented property across nearly all flavonoid subclasses (Forestryana et al., 2024).

Table I. Comparative phytochemical profile of balik angin leaves extracted with ethanol

Secondary metabolite	Ethanol extract source	
	Maceration	Soxhlet
Flavonoids	+	+
Alkaloids	+	+
Phenol	+	+
Tannins	+	+
Saponins	+	+
Triterpenoids	+	+
Steroids	-	-

The total phenolic content (TPC) of the ethanolic extract of balik angin leaves was quantified using the Folin–Ciocalteu assay, which is based on oxidation–reduction reactions. Gallic acid was selected as the reference standard due to its widespread occurrence in plants, high stability, and classification as a simple phenolic acid derived from hydroxybenzoic acid. Phenolic constituents form a yellow complex upon reaction with the Folin–Ciocalteu reagent in this assay; the subsequent addition of sodium carbonate (Na_2CO_3) generates an alkaline environment that converts the reaction mixture to a blue color. This color transformation occurs because phenolic compounds react with the Folin–Ciocalteu reagent under alkaline conditions, enabling proton release and the formation of phenolate ions (Ramadhan et al., 2021; Yuliani et al., 2022). Quantification of total phenolics was performed using the calibration equation $y = 0.0109x - 0.2316$.

In parallel, the total flavonoid content (TFC) of the ethanolic extract was determined using quercetin as the standard compound, with absorbance measured at 415 nm. Quercetin was chosen because it belongs to the flavonol subclass and readily forms a yellow-colored complex in the presence of aluminum chloride ($AlCl_3$) and glacial acetic acid (Marjoni et al., 2018). The flavonoid concentration was calculated from the quercetin standard curve described by the equation $y = 0.0062x - 0.002$.

Ahmed et al. (2019) reported that methanolic extracts of balik angin leaves contained total phenolic and flavonoid

levels of 25.23 ± 0.15 µg GAE/mg extract and 9.84 ± 0.06 µg QE/mg extract, respectively, both of which were lower than the values obtained in the present study. Such variation suggests that the chemical composition of plant extracts may differ depending on factors such as geographical origin and extraction conditions (Yahyaoui et al., 2017). In this study, the total flavonoid content of the ethanolic extract prepared using the Soxhlet method was generally higher than that reported by Ahmed et al. (2019), whereas the extract obtained through maceration exhibited a comparatively lower flavonoid concentration (Table II). Although Soxhlet extraction is widely recognized for its efficiency in recovering bioactive compounds, including antioxidants, previous investigations have demonstrated that this technique may yield higher total flavonoid levels under certain experimental conditions (Isnindar et al., 2025).

Table II. Comparative phenolic and flavonoid compounds of balik angin leaves extracted with ethanol

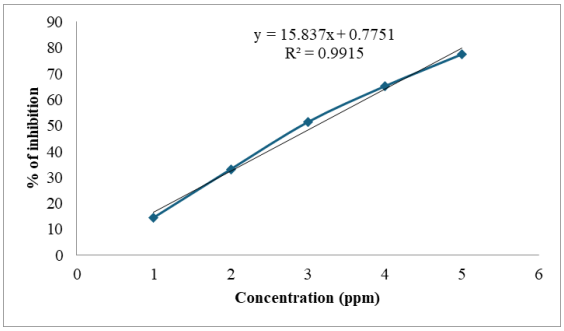
Extraction method	Value	
	TPC (µg GAE/mg)	TFC (µg QE/mg)
Maceration	381.2 ± 0.353	7.29 ± 0.019
Soxhlet	438.3 ± 0.122	11.64 ± 0.034

Soxhlet extraction often produces higher total phenolic content (TPC) than cold extraction methods such as maceration because it combines continuous hot solvent extraction, enhanced mass transfer, and repeated solvent recycling, which collectively increase the recovery of phenolic compounds from plant matrices. One of the main mechanisms explaining the higher phenolic yield in Soxhlet extraction is temperature-enhanced molecular diffusion. Elevated temperature increases the kinetic energy of molecules, accelerating the movement of phenolic compounds from plant cells into the solvent phase. Heating during Soxhlet extraction also enhances solvent penetration into plant tissues. When plant material is exposed to hot solvent at the solvent's boiling point, the cell walls and membranes become more permeable. This structural weakening allows the solvent to infiltrate intracellular spaces and dissolve bound phytochemicals more effectively (Osorio-Tobón, 2020).

Flavonoids play a major role in the antioxidant capacity of many medicinal plants by neutralizing free radicals through metal-binding and electron- or hydrogen-donation mechanisms under both in vivo and in vitro conditions (Figura & Fatokun, 2025). Numerous analytical approaches have been developed to assess the antioxidant potential of phenolic and flavonoid compounds, among which the DPPH (2,2-diphenyl-1-picrylhydrazine) assay is the most widely applied. DPPH is

a chemically stable radical commonly employed to measure the radical-scavenging activity of plant-derived extracts. This assay can be performed on either solid or liquid samples and does not target a specific antioxidant compound. The DPPH method is based on the ability of antioxidants to donate hydrogen atoms, leading to the reduction of the DPPH radical into its non-radical form, 2,2-diphenyl-1-picrylhydrazine. This reduction is visually observed as a color transition from deep purple to pale yellow, which serves as the analytical basis of the present study. The magnitude of this color change reflects the free radical scavenging effectiveness of the tested sample (Alsaleem et al., 2020; Baliyan et al., 2022).

The DPPH radical inhibition assay was quantitatively employed to evaluate the capacity of the tested extracts and the reference compound, quercetin, to neutralize DPPH radicals through hydrogen atom transfer or electron donation mechanisms resulting from interactions with the DPPH molecule. The IC_{50} parameter represents the concentration required to reduce 50% of DPPH radicals and serves as an indicator of antioxidant potency, with lower IC_{50} values corresponding to stronger antioxidant activity. Extracts exhibiting IC_{50} values below 50 ppm are considered to possess high free radical scavenging potential (Yunitrianti et al., 2020).



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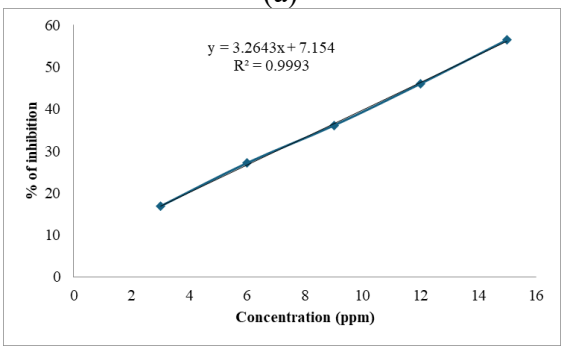

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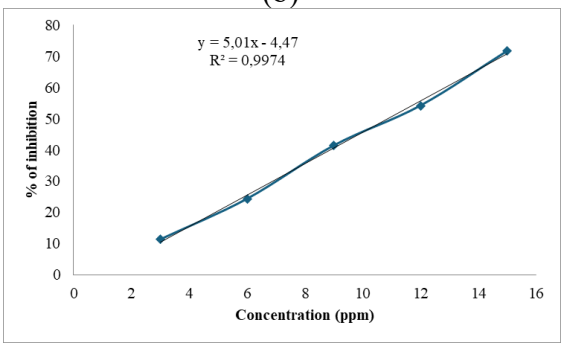

(c)

Figure 1. Regression equation curve of antioxidant activity test results: (a) quercetin, (b) macerated extract, and (c) soxhlet extract

Quercetin was selected as the standard comparator in this study due to its classification as a flavonol-type flavonoid, characterized by the presence of a carbonyl group at the C-4 position and hydroxyl substituents at the C-3 or C-5 positions, structural features that contribute to its antioxidant activity (Frenț et al., 2024). In

addition, flavonoid glycosides derived from quercetin and isorhamnetin have been reported in the stems of *Alphitonia philippinensis* (Al Omar et al., 2022). The calibration curve generated for quercetin demonstrated good linearity, expressed by the regression equation y = 15.387x + 0.7751 (Figure 1a), which was used to determine the IC_{50} value. Based on the assay results, quercetin exhibited very strong antioxidant activity, with an IC_{50} of 3.108 ppm.

As presented in Table III, the DPPH radical scavenging assay revealed a linear relationship for the ethanolic extract of balik angin leaves obtained through maceration, expressed by the regression equation y = 3.2643x + 7.154 (Figure 1b). Based on this equation, the calculated IC_{50} value was 13.126 ppm, indicating very strong antioxidant activity. In contrast, the ethanolic extract prepared using the Soxhlet technique showed a regression equation of y = 5.01x − 4.47 (Figure 1c), corresponding to an IC_{50} value of 10.872 ppm, which also falls within the very strong antioxidant classification. Overall, the IC_{50} values derived from the antioxidant assays demonstrate that both extracts, as well as the reference standard, possess very strong radical scavenging capacity, despite observable differences in their respective IC_{50} values. The assay further confirmed that quercetin, used as the positive control, exhibited the greatest ability to neutralize DPPH radicals. Among the plant extracts, the Soxhlet-derived sample displayed higher antioxidant activity than the extract obtained by maceration.

The antioxidant activity data of the macerated and Soxhlet extracts were first evaluated for normality using the Shapiro–Wilk test. The results showed significance values of 0.310 for the macerated extract, 0.215 for the Soxhlet extract, and 0.072 for the quercetin standard curve, all of which were greater than 0.05, indicating that the data were normally distributed. Subsequently, a homogeneity test was performed using Levene's test, which yielded a significance value of 0.000, indicating that the assumption of homogeneity was not satisfied (sig < 0.05). Therefore, a non-parametric comparison was conducted using the Kruskal–Wallis test. The Kruskal–Wallis analysis produced a significance value of 0.170 ($p > 0.05$), indicating that there was no statistically significant difference between the ethanolic leaf extracts obtained through maceration and Soxhlet extraction methods.

Table III. Comparative DPPH scavenging activity results of balik angin leaves extracted with ethanol

Samples	DPPH scavenging activity	
	Regression equation	**IC_{50} (ppm)**
Quercetin	y = 15.837x + 0.7751	3.108
Macerated extract	y = 3.2643x + 7.154	13.126
Soxhlet extract	y = 5.01x – 4.47	10.872

Table IV. SPSS test results of antioxidant activity

	Normality test	Homogeneity test	Kruskall - Wallis test
Quercetin (control)	0.072	0.000	0.170
Macerated extract	0.310		
Soxhlet extract	0.215		

Table V. Comparative CUPRAC activity results of balik angin leaves extracted with ethanol

Samples	CUPRAC activity	
	Regression equation	**EC_{50} (ppm)**
Quercetin	y = 7.8063x + 40.544	1.2113
Macerated extract	y = 3.3407x + 3.11	14.0359
Soxhlet extract	y = 3.0548x + 29.904	6.578

Antioxidant potential was further assessed using the CUPRAC and FRAP assays. The Cupric Ion Reducing Antioxidant Capacity (CUPRAC) method is based on the ability of antioxidant compounds to convert Cu^{2+} ions into Cu^{+} ions, a process that is visually indicated by a color transition from blue to yellow. In contrast, the Ferric Reducing Antioxidant Power (FRAP) assay evaluates the reduction of Fe^{3+} to Fe^{2+}, which is accompanied by a color change from green to bluish-green. Both techniques quantify antioxidant performance by correlating radical-scavenging capacity with the reducing power of the tested compounds (Munteanu & Apetrei, 2021).

Quercetin was employed as the reference standard in the CUPRAC assay due to its exceptionally strong antioxidant properties, reported to be four to five times more effective than vitamins C and E and approximately twice as potent as other flavonoid derivatives (Fawwaz et al., 2023). The high reducing ability of quercetin toward cupric ions was confirmed in this study, placing it in the very strong antioxidant category with an EC_{50} value of 1.2113 ppm. Both quercetin and ascorbic acid are known to participate in redox reactions by chelating metal ions, thereby diminishing their catalytic activity (Mendoza-Wilson et al., 2022). This metal-chelating action limits hydroxyl radical formation, which consequently reduces lipid peroxidation and DNA damage (He et al., 2017).

As summarized in Table V, ethanolic extracts of balik angin leaves obtained via Soxhlet extraction demonstrated greater cupric ion–reducing capacity than those produced by maceration. The Soxhlet-derived extract exhibited a lower EC_{50} value (6.578 ppm) compared with the macerated extract (14.0359 ppm), indicating stronger antioxidant activity. This enhanced reducing power is likely associated with the higher total phenolic content present in the Soxhlet extract relative to the maceration product.

The findings of this research indicate promising prospects for the development of balik angin leaves as a source of traditional antioxidant-based therapeutics. The ethanolic extract exhibited markedly stronger DPPH radical scavenging activity than that reported in earlier work by Ahmed et al. (2019), who documented an IC_{50} value of 32 ppm. Moreover, the extract demonstrated superior effectiveness in cupric ion reduction when compared with aqueous extracts prepared by infusion (EC_{50} of 17.76 ppm) and maceration (EC_{50} of 44.71 ppm), as previously reported by Ramadhan et al. (2024). Variations in phytochemical yield and biological activity during plant extraction are influenced by multiple parameters, including solvent selection and concentration, extraction technique, and operational temperature (Sun et al., 2025).

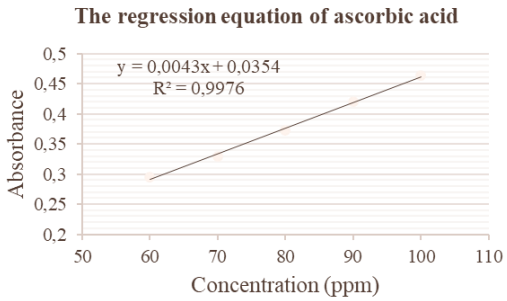

Figure 2. Ascorbic acid calibration curve

Antioxidant capacity measured by the FRAP assay was quantified using the ascorbic acid calibration curve expressed by the regression equation y = 0.0043x + 0.0354 (Figure 2), after which results were converted into milligrams of ascorbic acid equivalent per gram of extract (mg AAE/g). The reducing ability of ferric ions for the macerated extract reached 1,295.8 mg AAE/g extract, whereas the Soxhlet-derived extract demonstrated a higher value of 1,484.1 mg AAE/g extract. These findings further confirm that Soxhlet extraction is more effective at recovering antioxidant constituents from balik angin leaves, as indicated by its greater FRAP antioxidant capacity compared with the maceration method.

When compared with previous studies, the ethanolic extracts of balik angin leaves (*Alphitonia incana* (Roxb.) Teijsm. & Binn. ex Kurz) obtained in this work through both maceration and Soxhlet techniques exhibited substantially higher

ascorbic acid equivalent values. Ramadhan et al. (2024) reported that aqueous extracts prepared by maceration and infusion produced antioxidant activities of only 635 ± 0.5 mg AAE/g extract and 726 ± 0.8 mg AAE/g extract, respectively. In addition, the antioxidant strength observed in this study exceeded that reported for *Alphitonia philippinensis* extracted with methanol using the Soxhlet method, which yielded 9.36 ± 0.05 mg AAE/g extract (Ahmed et al., 2019).

Supporting these results, a review by Mungwari et al. (2025) emphasized that the Soxhlet technique is highly efficient for isolating bioactive compounds, including antioxidants, from natural sources. This method operates through continuous solvent heating and condensation, allowing repeated extraction cycles that enhance the recovery of solvent-soluble compounds from plant matrices.

The higher antioxidant performance observed in the Soxhlet extract was associated with its relatively greater phenolic and flavonoid contents. The higher the phenol and flavonoid content, the stronger the antioxidant activity. Phenolic compounds are widely recognized as potent natural antioxidants due to their ability to neutralize reactive oxygen species (ROS) and interrupt oxidative chain reactions. Phenolic compounds exert antioxidant activity through multiple complementary mechanisms, including hydrogen atom transfer (HAT), single electron transfer (SET), and transition metal chelation. Their effectiveness is largely determined by structural characteristics such as hydroxyl group number, aromatic conjugation, and electron delocalization. These mechanisms collectively enable phenolics to neutralize reactive oxygen species, interrupt radical chain reactions, and reduce oxidative stress in biological systems (Ahmad et al., 2025).

IV.CONCLUSION

The findings of this study indicate that Soxhlet extraction is more effective than maceration for obtaining antioxidant compounds from balik angin leaves. The ethanolic extract produced by the Soxhlet method contained higher levels of total flavonoids and phenolics (TFC: 11.64 ± 0.034 µg QE/mg; TPC: 438.3 ± 0.122 µg GAE/mg) compared with the extract obtained through maceration (TFC: 7.29 ± 0.019 µg QE/mg; TPC: 381.2 ± 0.353 µg GAE/mg). Consistently, the Soxhlet ethanolic extract showed stronger antioxidant capacity than the macerated extract in the DPPH, CUPRAC, and FRAP assays. However, statistical analysis indicated that the difference in antioxidant activity between the two extraction methods was not significant ($p > 0.05$).

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this research.

ACKNOWLEDGEMENT

The authors would like to acknowledge the the Ministry of Education and Culture, Research and Technology of the Republic of Indonesia for the financial support (Beginner Lecturer Research Grant 2024/Penelitian Dosen Pemula 2024) and the University of Borneo Lestari for providing the laboratory facility.

REFERENCES

Ahmad, Z., Rauf, A., Orhan, I. E., Mubarak, M. S., Akram, Z., Islam, M. R., Imran, M., Edis, Z., Kondapavuluri, B. K., Thangavelu, L., & Thiruvengadam, M. (2025). Antioxidant Potential of Polyphenolic Compounds, Sources, Extraction, Purification and Characterization Techniques: A Focused Review. *Food Science and Nutrition*, *13*(12). https://doi.org/10.1002/fsn3.71259

Ahmed, J., Salim, K. A., Lim, L. B. L., & Jama, A. M. (2019). Evaluation of antioxidant activity and phytochemical screening of leaves, barks, stems and fruits of *Alphitonia philippinensis* (Rhamnaceae) from Brunei Darussalam. *Pharmacognosy Journal*, *11*(5), 951–961. https://doi.org/10.5530/pj.2019.11.151

Al Omar, R., Micklewright, R., Masud, K., Naz, T., Vemulpad, S., & Jamie, J. (2022). The genus Alphitonia Reissek ex Endl. (Rhamnaceae): A review of its customary uses, phytochemistry and biological activities. *Journal of Ethnopharmacology*, *294*, 115168. https://doi.org/10.1016/j.jep.2022.115168

Alsaleem, K. A., Elfaruk, M. S., & Hammam, A. R. A. (2020). Estimation of antioxidants activity and capacity to capture free radical using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. *Australian Journal of Basic and Applied Sciences*, *14*(11), 86–93. https://doi.org/10.22587/ajbas.2020.14.11.9

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*, *27*(4). https://doi.org/10.3390/molecules27041326

Cao, S., Liang, J., Chen, M., Xu, C., Wang, X., Qiu, L., Zhao, X., & Hu, W. (2025). Comparative analysis of extraction technologies for plant extracts and absolutes. *Frontiers in Chemistry*, *13*, 1–16. https://doi.org/10.3389/fchem.2025.1536590

Cock, I. E. (2020). *Alphitonia excelsa* (Fenzl) Benth. Leaf Extracts Inhibit the Growth of a Panel of Pathogenic Bacteria. *Pharmacognosy Communications*, *10*(2), 67–74. https://doi.org/10.5530/pc.2020.2.14

Fawwaz, M., Pratama, M., Musafira, M., Wahab, I., Iriani, R., Aminah, A., Kusuma, A. T., & Arsul, M. I. (2023). Evaluation of Antioxidant Activity of *Vernonia amygdalina* Leaves and Its Flavonoid-Phenolic Content. *Indonesian Journal of Pharmaceutical Science and Technology*, *10*(2), 104. https://doi.org/10.24198/ijpst.v10i2.41617

Figura, M., & Fatokun, A. A. (2025). Flavonoids and related compounds targeting pathomechanisms of Parkinson's Disease – from bench to

bedside: A focused review of current perspectives, clinical trial outcomes, and future directions. *Phytomedicine Plus*, *5*(4), 100861. https://doi.org/10.1016/j.phyplu.2025.100861

Forestryana, D., Pebrianie, S., Ramadhan, H., & Restapaty, R. (2024). Formulation of Wound Healing Hydrogel from 70% Ethanol Extract of Kelakai Roots (*Stenochlaena palustris* (Burm. F.) Bedd) with Polymer Combination of PVA/HPMC. *Journal of Pharmaceutical Sciences and Community*, *21*(1), 23–31. https://doi.org/10.24071/jpsc.005291

Forestryana, D., Ramadhan, H., Sayakti, P. I., Nurjanah, T., & Faradillah, N. (2022). Identification Of Essential Oils From The Bark Of Balik Angin (*Alphitonia Incana* (Roxb.) Teijsm. & Binn. Ex Kurz). *Indonesian Journal of Pharmaceutical Science and Technology*, *1*(1), 85–95. https://doi.org/10.24198/ijpst.v1i1.42498

Frenț, O. D., Stefan, L., Morgovan, C. M., Duteanu, N., Dejeu, I. L., Marian, E., Vicaș, L., & Manole, F. (2024). A Systematic Review: Quercetin—Secondary Metabolite of the Flavonol Class, with Multiple Health Benefits and Low Bioavailability. *International Journal of Molecular Sciences*, *25*(22), 1–47. https://doi.org/10.3390/ijms252212091

Fuentes, R. G., Valenciano, A. L., Cassera, M. B., & Kingston, D. G. I. (2020). Antiproliferative and antiplasmodial investigation of *Alphitonia excelsa* and *Arcangelesia flava*. *Philippine Journal of Science*, *149*(1), 115–120. https://doi.org/10.56899/149.01.12

Gulcin, İ. (2025). Antioxidants: a comprehensive review. *Archives of Toxicology, 99*(5), 1893–1997. https://doi.org/10.1007/s00204-025-03997-2

Hasnaeni, H., Wisdawati, W., & Usman, S. (2019). Pengaruh Metode Ekstraksi Terhadap Rendemen Dan Kadar Fenolik Ekstrak Tanaman Kayu Beta-Beta (*Lunasia amara* Blanco). *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *5*(2), 175–182. https://doi.org/10.22487/j24428744.2019.v5.i2.13149

He, L., He, T., Farrar, S., Ji, L., Liu, T., & Ma, X. (2017). Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. *Cellular Physiology and Biochemistry*, *44*(2), 532–553. https://doi.org/10.1159/000485089

Hidayati, S. (2025). Perbandingan Metode Ekstraksi Sokhletasi dan Maserasi Terhadap Aktivitas Antioksidan Ekstrak Daun Ciplukan (*Physalis angulata*). *Pharmacon*, *14*(2), 960–967. https://doi.org/10.35799/pha.14.2025.57719

Isnindar, I., Luliana, S., & Zahid, M. (2025). Effect of extraction, ratio, and solvent concentration on total flavonoid content and antioxidant activit of singkel (*Premna serratifolia* linn.) using DPPH method. *Jurnal Teknosains*, *14*(2), 103. https://doi.org/10.22146/teknosains.100099

Kapoor, B., Bhardwaj, S., Singh, R., Sharma, A., Dolma, Y., Sharma, A., Sharma, N. R., Landi, M., & Kapoor, D. (2025). Multifaceted role of plant polyphenols in human health: a comprehensive review. *Food Science and Biotechnology*, *0123456789*. https://doi.org/10.1007/s10068-025-01991-z

Ki, M. R., Youn, S., Kim, D. H., & Pack, S. P. (2024). Natural Compounds for Preventing Age-Related Diseases and Cancers. *International Journal of Molecular Sciences*, *25*(14), 7530. https://doi.org/10.3390/ijms25147530

Marjoni, M. R., Nofita, D., Rahmi, N., Saifullah, & Najla, N. A. (2018). Phenolics compounds, flavonoids, and antioxidant activity methanol extract of arum manis leaves (*Mangifera indica* L. Var. Arumanis). *International Journal of Green Pharmacy*, *12*(3), S651–S656. https://doi.org/10.22377/ijgp.v12i03.2034

Mendoza-Wilson, A. M., Balandrán-Quintana, R. R., Valdés-Covarrubias, M. Á., & Cabellos, J. L. (2022). Potential of quercetin in combination with antioxidants of different polarity incorporated in oil-in-water nanoemulsions to control enzymatic browning of apples. *Journal of Molecular Structure*, *1254*, 132372. https://doi.org/10.1016/j.molstruc.2022.132372

Mungwari, C. P., King'ondu, C. K., Sigauke, P., & Obadele, B. A. (2025). Conventional and modern techniques for bioactive compounds recovery from plants: Review. *Scientific African*, *27*, e02509. https://doi.org/10.1016/j.sciaf.2024.e02509

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, *22*(7), 3380. https://doi.org/10.3390/ijms22073380

Münzel, T., Hahad, O., Sørensen, M., Lelieveld, J., Duerr, G. D., Nieuwenhuijsen, M., & Daiber, A. (2022). Environmental risk factors and cardiovascular diseases: a comprehensive expert review. *Cardiovascular Research*, *118*(14), 2880–2902. https://doi.org/10.1093/cvr/cvab316

Osorio-Tobón, J. F. (2020). Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology*, *57*(12), 4299–4315. https://doi.org/10.1007/s13197-020-04433-2

Purnama, S., Ramadhan, H., & Putri, I. S. (2022). Uji Aktivitas Antioksidan Fraksi N-Heksan dari Ekstrak Metanol Daun Binjai *Mangifera caesia* Jack. Ex. Wall Menggunakan Metode DPPH. *Jurnal Ilmu Kefarmasian Indonesia*, *20*(1), 55–62. https://doi.org/10.35814/jifi.v20i1.1133

Ramadhan, H., Andina, L., Vebruati, V., Nafila, N., Yuliana, K. A., Baidah, D., & Lestari, N. P. (2020). Phytochemical Screening And Randemen Comparison Of 96% Ethanol Extract Of Terap (*Artocarpus odoratissimus* Blanco) Leaf, Flesh And Peel. *Jurnal Ilmiah Farmako Bahari*, *11*(2), 103–112. https://doi.org/10.52434/jfb.v11i2.876

Ramadhan, H., & Forestryana, D. (2021). The effect of different extraction methods on the total phenolic content and antioxidant activity in galam sawdust (*Melaleuca leucadendron* linn.). *Tropical Journal of Natural Product Research*, *5*(5), 805–808. https://doi.org/10.26538/tjnpr/v5i5.2

Ramadhan, H., Forestryana, D., Adisaputra, G. T., & Muharram, S. R. (2022). Effect of Vanillin Quality on the Synthesis and Solid Dispersion Systems on Pgv-0 Antioxidant Capacity. *International Journal of Applied Pharmaceutics*, *14*(Special Issue 2), 31–36. https://doi.org/10.22159/ijap.2022.v14s2.44746

Ramadhan, H., Forestryana, D., Muthia, R., Fitriyanti, Helmina, R., & Fahriana, Y. (2024). Formulasi Gel Ekstrak Metanol Daun Balik Angin (*Alphitonia incana* (Roxb .) Teijsm . & Binn . Ex Kurz). *Farmasains: Jurnal Ilmiah Ilmu Kefarmasian*, *11*(2), 98–113. https://doi.org/10.22236/farmasains.v11i2.9863

Ramadhan, H., Forestryana, D., Subareng, A. R. M., & Vebruati. (2025). The Effect of Different Soxhlet Extraction Solvents Towards Anti-*Propionibacterium acnes* Activity of Balik Angin Leaves (*Alphitonia incana* (Roxb.) Teijsm. & Binn. ex Kurz). *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *11*(1), 1–12. https://doi.org/10.22487/j24428744.2025.v11.i1.16634

Ramadhan, H., Forestryana, D., Sayakti, P. I., Susiani, E. F., Pambudi, D. R., Rosyada, A., Khairany, N., Izzatul, W., Dewi, W. A., & Syifa, N. (2025). Comparison of Maceration and Soxhlet Towards Phenolic-Flavonoid Content and Antioxidant Activity of Leaves Methanolic Extract of *Alphitonia Incana*. *Proceeding ICSNPH*, 57–69.

Ramadhan, H., Muthia, R., Wahyunita, S., Forestryana, D., Soleha, S. M., & Lihimi, L. (2023). Comparison of Extraction Solvents Towards Anti-*Propionibacterium acnes* activity of *Alphitonia incana* (Roxb). Teijsm. & Binn. ex Kurz Leaves. *Indonesian Journal of Pharmaceutical Science and Technology*, *SUPP1*(1), 10-19. https://doi.org/10.24198/ijpst.v0i0.45897

Ramadhan, H., Rezky, D. P., & Susiani, E. F. (2021). Penetapan Kandungan Total Fenolik-Flavonoid pada Fraksi Etil Asetat Kulit Batang Kasturi (*Mangifera casturi* Kosterman). *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *8*(1), 58–67. https://doi.org/10.20473/jfiki.v8i12021.58-67

Ramadhan, H., Susiani, E. F., Forestryana, D., Raflianti, D., Azizah, H. N., & Iedliany, F. (2024). Comparison of maceration and infundation towards antioxidant capacity of leaves aqueous extracts of balik angin (*Alphitonia incana* (Roxb.) Teijsm. & Binn. ex Kurz). *Pharmacy Education*, *24*(6), 7–14. https://doi.org/10.46542/pe.2024.246.714

Sayakti, P. I., Anisa, N., & Ramadhan, H. (2022). Antioxidant activity of methanol extract of cassava leaves (*Manihot esculenta* Crantz) using CUPRAC method. *Jurnal Ilmiah Farmasi*, 97–106. https://doi.org/10.20885/jif.specialissue2022.art12

Sun, S., Yu, Y., Jo, Y., Han, J. H., Xue, Y., Cho, M., Bae, S. J., Ryu, D., Park, W., Ha, K. T., & Zhuang, S. (2025). Impact of extraction techniques on phytochemical composition and bioactivity of natural product mixtures. *Frontiers in Pharmacology*, *16*, 1–14. https://doi.org/10.3389/fphar.2025.1615338

Sutomo, S., Arnida, A., Rizki, M. I., Triyasmono, L., Nugroho, A., Mintowati, E., & Salamiah, S. (2016). Skrining Fitokimia dan Uji Kualitatif Aktivitas Antioksidan Tumbuhan Asal Daerah Rantau Kabupaten Tapin Kalimantan Selatan. *Jurnal Pharmascience*, *3*(1), 66–74. http://dx.doi.org/10.20527/jps.v3i1.5836

Tumilaar, S. G., Hardianto, A., Dohi, H., & Kurnia, D. (2023). A Comprehensive Review of Free Radicals, Oxidative Stress, and Antioxidants: Overview, Clinical Applications, Global Perspectives, Future Directions, and Mechanisms of Antioxidant Activity of Flavonoid Compounds. *Journal of Chemistry*, *2024*. https://doi.org/10.1155/2024/5594386

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines*, *5*(3), 93. https://doi.org/10.3390/medicines5030093

Wardah, W., & Sundari, S. (2019). Ethnobotany study of Dayak society medicinal plants utilization in Uut Murung District, Murung Raya Regency, Central Kalimantan. *IOP Conference Series: Earth and Environmental Science*, *298*(1), 012005. https://doi.org/10.1088/1755-1315/298/1/012005

Yahyaoui, M., Ghazouani, N., Sifaoui, I., & Abderrabba, M. (2017). Comparison of the Effect of Various Extraction Methods on the Phytochemical Composition and Antioxidant Activity of *Thymelaea hirsuta* L. aerial parts in Tunisia. *Biosciences, Biotechnology Research Asia*, *14*(3), 997–1007. https://doi.org/10.13005/bbra/2534

Yuliani, C. R., Ramadhan, H., Sayakti, P. I., & Torizellia, C. (2022). Total phenolic and flavonoid contents of n-hexane fraction in binjai leaves (*Mangifera caesia* Jack. ex. Wall). *Jurnal Ilmiah Farmasi*, 11–19. https://doi.org/10.20885/jif.specialissue2022.art2

Yunitrianti, Elya, B., & Noviani, A. (2020). Determination of the antioxidant activity of prasman leaf extracts (*Ayapana Triplinervis* [VAHL]) and the total flavonoid and phenol contents of the most active extracts. *International Journal of Applied Pharmaceutics*, *12*(Special Issue 1), 107–111. https://doi.org/10.22159/ijap.2020.v12s1.FF021