



Phytochemical Screening and Antioxidant Activity of the Ethyl Acetate Fraction of Paliasa (*Kleinhovia hospita* L.) Stem Bark

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Abstract. *Paliasa* (*Kleinhovia hospita* Linn) is a medicinal plant that may contain secondary metabolites with antioxidant potential. This study evaluated the antioxidant activity of the ethyl acetate fraction of paliasa stem bark using the ABTS radical-scavenging assay. Dried stem-bark powder (300 g) was macerated with methanol, and the resulting extract was partitioned to obtain the ethyl acetate fraction. Phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, tannins, and a terpenoid/steroid-class reaction, although the latter identification requires confirmation. The ABTS assay was performed at concentrations of 20, 40, 60, 80, 100, and 120 ppm in triplicate. Mean radical inhibition increased with concentration, from 40.374% at 20 ppm to 54.536% at 120 ppm. The reported mean IC₅₀ value was 88.621 ppm, based on three determinations (87.486, 88.939, and 89.438 ppm). These results indicate concentration-dependent ABTS radical-scavenging activity of the ethyl acetate fraction under the tested conditions. Before publication, the ABTS measurement wavelength, reagent-preparation details, sample identity used for phytochemical screening, and fraction-yield calculation should be checked against the original laboratory records.

Keywords: *Kleinhovia Hospita*; *Paliasa*; Ethyl Acetate Fraction; Antioxidant Activity; ABTS

1. Introduction

The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the principal conclusions. As far as possible, please keep the introduction comprehensible to scientists outside your particular field of research. References should be numbered in order of appearance and indicated by a numeral or Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defense system to neutralize them. At excessive levels, ROS can oxidize lipids, proteins, and nucleic acids, potentially disrupting cellular function. Antioxidants can inhibit these processes through several mechanisms, including electron or hydrogen-atom donation, radical stabilization, and chelation of metal ions that catalyze oxidation reactions. However, antioxidant activity cannot be inferred from a single mechanism or assay result because the response is affected by compound structure, reaction conditions, solvent, and analytical

method. Therefore, screening natural sources of antioxidants requires measurable experimental methods and cautious interpretation.(Huang et al., 2005; Lobo et al., 2010; Munteanu & Apetrei, 2021; Pisoschi et al., 2016)

Plants are sources of diverse secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, and saponins. Several of these compound groups have been studied for their potential contribution to radical-scavenging activity, although detecting a metabolite class by qualitative phytochemical screening does not by itself prove that the class is the primary cause of a sample's biological activity. Phenolic compounds and flavonoids, for example, have hydroxyl groups that can participate in redox reactions, whereas plant-extract activity reflects interactions among mixtures of compounds with different concentrations and reactivities. Accordingly, phytochemical screening is a preliminary assessment of detected metabolite classes; identification of individual compounds requires further analytical techniques.(Dai & Mumper, 2010; Do et al., 2014; Gülçin, 2020; Gulcin, 2025; Rice-Evans et al., 1997)

One plant of interest is paliasa, *Kleinhovia hospita* L., which is used traditionally in Indonesia. Previous studies of *K. hospita* leaves have reported antioxidant activity in extracts and fractions using the DPPH method, including activity in the ethyl acetate fraction (Arung et al., 2009). Another study of paliasa leaves evaluated total phenolic and flavonoid contents and antioxidant activity (Nuralifah et al., 2022) , whereas a study of tahongai leaf infusions also reported DPPH radical-scavenging activity (Leswana & Sianturi, 2024). These findings support further research on different plant parts and preparations. However, results from leaves, infusions, crude extracts, or the DPPH method cannot be directly equated with results for an ethyl acetate stem-bark fraction tested using ABTS because plant part, chemical composition, extraction procedure, and test-radical characteristics can affect measurements.

Paliasa stem bark has also been investigated in natural-products chemistry. Dini and Darminto (2012) reported the isolation of compounds from *K. hospita* stem bark and identified indications of phenolic and alkaloid classes in the isolates obtained. More recent research on paliasa stem-bark extracts reported several secondary-metabolite classes and evaluated antioxidant activity using DPPH (Imran et al., 2025; Imran & Alwahab, 2024) . Meanwhile, research on purified paliasa leaf extract showed that antioxidant activity can change after purification and formulation; therefore, sample-processing steps should be considered when comparing activity values (Suryani et al., 2017). In general, solvent selection and extraction technique affect which compounds are extracted, because solvent polarity, contact time, temperature, and plant-matrix characteristics all contribute to extract composition (Azwanida, 2015; Chaves et al., 2020; Dai & Mumper, 2010).

Liquid-liquid fractionation is an approach for separating an extract mixture according to differences in compound distribution between two immiscible solvents. Ethyl acetate has intermediate polarity and can extract some semipolar components, including certain phenolic compounds, depending on system conditions and the chemical properties of each compound. Because a fraction is a complex mixture, its yield cannot serve as the sole measure of antioxidant potency. Activity should be measured directly, while phytochemical screening can provide an initial indication of the compound classes detected. Interpretation of extraction and fractionation results should account for the fact that different solvents can produce different phytochemical profiles and activities (Azwanida, 2015; Chaves et al., 2020; Do et al., 2014).

Radical-scavenging activity can be evaluated using several methods, including DPPH, ABTS, FRAP, and ORAC. Each method has a different reaction principle and analytical limitation; therefore, results from different methods are not always directly comparable (Abramovič et al., 2018; Apak et al., 2016; Cahyaningsih et al., 2021; Prior et al., 2005; Schaich et al., 2015; Tan & Lim, 2015). The ABTS method uses the ABTS•⁺ radical cation, which can be generated by oxidizing ABTS, for example with potassium persulfate. When an antioxidant reacts with this radical, the solution's color fades and the change in absorbance is used to estimate radical-scavenging capacity. The method introduced by (Re et al. (1999)) can be applied to various hydrophilic and lipophilic antioxidants, but results still depend on reagent concentration, incubation time, solvent, wavelength, and measurement conditions. Methodological reviews also emphasize the need to consider reaction kinetics and the limitations of colorimetric methods when interpreting antioxidant activity (Huang et al., 2005; Munteanu & Apetrei, 2021; Schaich et al., 2015).

The IC₅₀ value is often used to express the sample concentration that produces 50% inhibition under specified test conditions. A lower value indicates that a smaller sample concentration is required to achieve that response in the same test system. Nevertheless, IC₅₀ is an operational parameter that depends on experimental design, concentration range, regression model, and reaction time; it is not direct evidence of antioxidant efficacy in the body. Comparisons across studies should therefore consider the method, reference standard, concentration units, and analytical conditions used (Gulcin, 2025; Prior et al., 2005; Schaich et al., 2015; Tan & Lim, 2015). Replicate measurements and reporting data variability are also important so that activity estimates are not based on a single observation.

Based on the foregoing, this study aimed to qualitatively identify secondary-metabolite classes in the ethyl acetate fraction of paliasa stem bark (*Kleinhovia hospita* L.) and to evaluate its radical-scavenging activity using the ABTS method. The focus on the ethyl acetate stem-bark fraction and use of ABTS complement earlier studies, many of which examined paliasa leaves or used the DPPH method. The findings provide preliminary data on the fraction's phytochemical characteristics and antioxidant activity, while supporting further research to identify active compounds more specifically. This exploratory work is relevant to SDG 3 (Good Health and Well-Being) through preliminary research on plant-derived antioxidant activity and to SDG 12 (Responsible Consumption and Production) through the investigation of a plant resource; however, the present in-vitro assay does not establish clinical benefit or demonstrate sustainability outcomes.

2. Methods

2.1. Materials and Instruments

The materials used in this study included paliasa stem bark (*Kleinhovia hospita* L.), methanol, n-hexane, ethyl acetate, ethanol, distilled water, ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], potassium persulfate (K₂S₂O₈), hydrochloric acid (HCl), magnesium, chloroform, acetic anhydride, concentrated sulfuric acid, iron(III) chloride (FeCl₃), and Dragendorff reagent. Ascorbic acid was listed as a reference material in the source procedure; quantitative reference-standard results are not included in the dataset presented here.

The equipment used included a rotary evaporator, UV-Vis spectrophotometer, blender, analytical balance, separatory funnel, beakers, Erlenmeyer flasks, graduated

cylinders, volumetric pipettes, Pasteur pipettes, volumetric flasks, test tubes, and other laboratory glassware.

2.2. Sample Preparation and Extraction

Paliassa stem bark was cut into small pieces and then sun-dried for 20 days. The dried sample was ground in a blender to obtain powdered crude drug material. A 300 g portion of powdered crude drug was macerated in methanol for 48 hours. The residue was then macerated again for 48 hours using the same solvent. The filtrates from both maceration stages were combined and evaporated using a rotary evaporator to obtain a concentrated methanol extract.

2.3. Liquid-Liquid Fractionation

Fractionation was performed using liquid-liquid partitioning in a separatory funnel. A 250 mL portion of methanol macerate was transferred to a separatory funnel and partitioned with 50 mL portions of n-hexane at each stage. The mixture was gently shaken, allowed to stand until two layers formed, and then separated. The process was repeated until the n-hexane layer appeared clear.

The remaining macerate was then partitioned with ethyl acetate using a similar procedure. The ethyl acetate fractions were collected and evaporated using a rotary evaporator to obtain a concentrated fraction. The resulting fraction was weighed to determine the yield and then used for phytochemical and antioxidant activity assays.

The fraction yield was calculated as the mass of the ethyl acetate fraction relative to the starting extract mass subjected to fractionation, using the following equation:

$$\text{Yield (\%)} = (\text{fraction mass} / \text{initial extract mass}) \times 100$$

2.4. Phytochemical Screening

Phytochemical screening was conducted qualitatively using color reactions or precipitate formation to identify secondary-metabolite classes in the ethyl acetate fraction of paliassa stem bark.

The alkaloid test was performed by adding two drops of Dragendorff reagent to 1 mL of filtrate. Formation of an orange to orange-red precipitate was considered a positive result. The flavonoid test was performed by adding 5 mg of magnesium chloride to 2 mL of sample, allowing it to stand for one minute, and then adding 10 drops of concentrated HCl. A color change to red or yellowish brown was recorded as an indication of flavonoids.

The saponin test was performed by adding 10 mL of hot water to 44.3 mg of sample. The mixture was shaken, allowed to stand for 10 minutes, and then examined for foam formation. Next, two drops of 1 N HCl were added, and the mixture was shaken vigorously for one minute to assess foam stability.

The terpenoid/steroid screening procedure used 4.9 mg of sample dissolved in 2 mL of chloroform. The solution was placed dropwise on a spot plate and allowed to evaporate; 10 drops of acetic anhydride and three drops of concentrated H₂SO₄ were then added. The source procedure describes a red reaction as indicative of triterpenoids and a blue or green reaction as indicative of steroids. The tannin test was performed by adding 15 mL of hot water to 45.6 mg of sample, heating the mixture to boiling for five minutes, and filtering it. Several

drops of 10% FeCl₃ were added to the filtrate; formation of a dark color was recorded as a positive result for tannins.

2.5. ABTS Radical Scavenging Assay

A 1,000 ppm stock solution of the ethyl acetate fraction was prepared by dissolving 50 mg of sample in ethanol and making the volume up to 50 mL. The source thesis reports an ABTS concentration of 0.007 M and incubation of the ABTS/potassium persulfate mixture for 12–16 hours in the dark to generate ABTS•⁺ radicals. The exact reagent masses, stock-solution volumes, mixing ratio, and final working-solution absorbance must be checked against the laboratory record before this procedure can be considered fully reproducible.

The maximum absorption wavelength of the ABTS solution was reportedly scanned over 400–800 nm using a UV-Vis spectrophotometer. The sample stock solution was diluted to 20, 40, 60, 80, 100, and 120 ppm. A 0.1 mL portion of each test solution was mixed with 2 mL of ABTS solution and 4 mL of ethanol. The mixture was allowed to stand for 30 minutes at room temperature, and absorbance was measured in triplicate. The source thesis gives 750 nm in the Methods section but 734 nm in the Discussion; the actual wavelength used must be confirmed from the instrument record and made consistent throughout the manuscript.

2.6. Data Analysis

ABTS radical-scavenging activity was expressed as percent inhibition, calculated using the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_s) / A_0] \times 100$$

where A_0 is the absorbance of the blank and A_s is the absorbance of the sample. The IC₅₀ value was determined from the relationship between sample concentration and percent inhibition using an appropriate regression model. IC₅₀ is defined as the sample concentration that produces 50% radical inhibition. A lower IC₅₀ value indicates greater radical-scavenging activity under the same test conditions.

3. Results and Discussion

3.1. Extraction and Fractionation Yields

A total of 300 g of powdered paliasa stem bark was extracted with methanol through two maceration stages, each lasting 48 hours. The thesis reported a methanol-extract yield of 0.964%, corresponding to approximately 2.892 g of concentrated extract (300 g × 0.964%), which was used as the starting mass for fractionation. Liquid-liquid fractionation yielded 1.022 g of ethyl acetate fraction. Based on these reported masses, the calculated fraction yield is approximately 35.34% (1.022/2.892 × 100); the original weighing records should be checked before finalizing the reported value.

Table 1. Masses and Yield of the Ethyl Acetate Fraction from Paliasa Stem Bark		
Parameter	Reported value	Description
Initial powdered crude drug	300 g	K. hospita L. stem bark
Methanol-extract yield	0.964%	Reported in the thesis; equivalent to approximately 2.892 g from 300 g
Starting extract mass for fractionation	2.892 g	Basis for calculating fraction yield
Ethyl acetate fraction mass	1.022 g	After solvent evaporation
Ethyl acetate fraction yield	35.34%	$(1,022/2,892) \times 100\%$

As shown in Table 1, the calculated fraction yield is approximately 35.34% of the extract mass subjected to fractionation under the reported working conditions. Yield does not directly indicate antioxidant-compound content or chemical purity. The fraction remains a mixture and requires further characterization before any specific compound can be causally linked to antioxidant activity. (Michalaki & Grintzalis, 2023)

The source thesis reports a yield of 35.33%, whereas calculation from the rounded masses provided (1.022 g and 2.892 g) gives 35.34% when rounded to two decimal places. The value should be finalized after checking the unrounded weighing records; Table 1 currently shows the calculation based on the reported masses.

3.2. Phytochemical Screening Results

Qualitative screening of the ethyl acetate fraction was reported as positive for alkaloids, flavonoids, saponins, and tannins. The additional test was labeled “terpenoid” in the source table, but the recorded green color was interpreted there as steroid. Because the source procedure associates a red reaction with triterpenoids and a blue/green reaction with steroids, this result is provisionally described as a steroid-indicative reaction rather than confirmed evidence of terpenoids. The original observation and sample identity should be checked against the laboratory notes.

Table 2. Qualitative Phytochemical Screening of the Ethyl Acetate Fraction from Paliasa Stem Bark

Class in source table	Intensity	Reported observation	Interpretive note
Alkaloid	+++	Deep brick-red color	Qualitatively positive
Flavonoid	++	Yellowish brown	Qualitatively positive
Saponin	++	Foam formed	Qualitatively positive
Tannin	+++	Deep greenish-black	Positive according to the source criteria
Steroid-indicative reaction (provisional)	+	Green color (as recorded in source table)	Consistent with the source procedure’s steroid indication; confirm original observation

Table 2 provides preliminary qualitative indications of metabolite classes, not structural identification or quantification. Color reactions and foam formation can be affected by the matrix, concentration, reagents, and test conditions. The symbols +, ++, and +++

represent the visual intensity reported in the source and should not be interpreted as validated measures of concentration (Michalaki & Grintzalis, 2023).

The presence of flavonoids and tannins could plausibly contribute to radical scavenging through electron or hydrogen-atom transfer, but screening results alone do not prove that either class is the primary cause of ABTS activity. The ABTS response depends on antioxidant structure and reaction pathway; some phenolic compounds can form addition products with ABTS•+, whereas others react through oxidation without forming an adduct (Ilyasov et al., 2020)

These findings are broadly consistent with a recent report on paliasa stem bark that detected alkaloids, flavonoids, tannins, and saponins in the ethyl acetate fraction, although that study used DPPH rather than ABTS. IC₅₀ values from different methods cannot be compared as fully equivalent measures because reaction conditions and radical chemistry differ (Ilyasov et al., 2020; Imran & Alwahab, 2024; Munteanu & Apetrei, 2021).

3.3. ABTS Radical-Scavenging Activity

The assay was performed at six concentrations of the ethyl acetate fraction – 20, 40, 60, 80, 100, and 120 ppm with three replicates. Percent inhibition increased as concentration increased. Individual inhibition values and the recalculated means and standard deviations are presented in Table 3.

Table 3. ABTS Radical-Scavenging Activity of the Ethyl Acetate Fraction at Different Concentrations

Concentration (ppm)	Inhibition 1 (%)	Inhibition 2 (%)	Inhibition 3 (%)	Mean (%)	SD
20	40.634	40.374	40.115	40.374	0.260
40	43.660	43.534	43.579	43.591	0.064
60	46.254	46.695	46.320	46.423	0.238
80	48.271	48.420	48.629	48.440	0.180
100	51.297	51.580	51.082	51.320	0.250
120	54.323	54.741	54.545	54.536	0.209

In Table 3, mean inhibition increased from 40.374% at 20 ppm to 54.536% at 120 ppm. This increase is consistent with a concentration–effect response across the tested range. At 80 ppm, mean inhibition remained below 50%, whereas at 100 ppm it exceeded 50%; therefore, the 50% crossing lies between these two concentrations. This pattern supports using concentration-versus-inhibition regression to estimate IC₅₀, noting that the regression value depends on the model and data used.

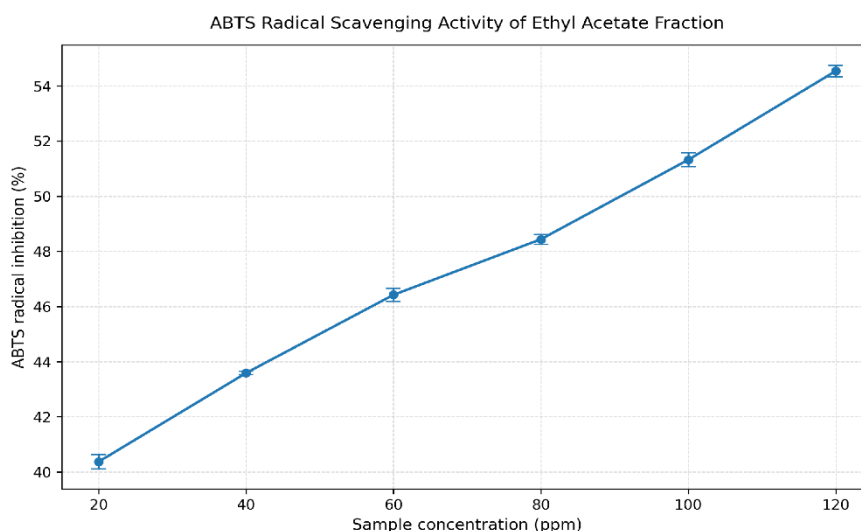


Figure 1. Mean ABTS radical-scavenging activity of the ethyl acetate fraction across the tested concentrations

As shown in Figure 1, the ABTS•+ method is based on the decrease in absorbance of the colored radical after reaction with an antioxidant. Although this method is widely used, results can vary with radical-generation procedures, solvent, incubation time, wavelength, and reagent-volume ratios. Methodological reviews emphasize the need to control test conditions and not to assume that results from different ABTS protocols are automatically comparable (Ilyasov et al., 2020; Munteanu & Apetrei, 2021).

The source thesis is internally inconsistent regarding measurement wavelength: its Methods section specifies 750 nm, whereas its Discussion specifies 734 nm. The wavelength actually used in the experiment must be confirmed from the spectrophotometer record and then reported consistently in Sections 2.5 and 3.3. Neither value should be selected solely because it is commonly used in other ABTS protocols.

The source thesis also contains an ambiguity in the ABTS reagent preparation: it describes two solutions made up to 100 mL, but then states that the mixture was diluted to 25 mL. These details cannot be reconciled reliably from the thesis text alone. The original laboratory record must be checked for the actual reagent masses, volumes, mixing ratio, and absorbance of the working solution before the method is finalized.

3.4. IC₅₀ Value and Activity Category

Table 4 shows IC₅₀ values for the three replicates of 89.438, 87.486, and 88.939 ppm, with a mean of 88.621 ppm. According to the classification used in the primary source, values of 50–100 ppm are categorized as strong antioxidant activity; accordingly, an IC₅₀ of 88.621 ppm falls within the strong category under that scheme. Such categories are operational conventions, not universal thresholds applicable to all methods, radicals, or matrices.

Table 4. IC₅₀ Values of the Ethyl Acetate Fraction of Paliassa Stem Bark

Replicate	IC ₅₀ (ppm)
1	89.438
2	87.486
3	88.939
Reported mean	88.621

The IC₅₀ value should be interpreted as the estimated concentration that produces 50% inhibition under the test conditions used, not as direct evidence of biological efficacy in the body. The ABTS assay is an in vitro chemical test; radical-scavenging capacity in a model system does not necessarily demonstrate bioavailability, safety, or therapeutic effects. The antioxidant-assessment literature recommends limiting interpretation to the method and test system, and using complementary approaches when making broader biological claims (Nwozo, 2023).

For context, a previous study reported an IC₅₀ of 51.7687 ppm for the ethyl acetate fraction of paliassa stem bark using the DPPH method. That value is lower than the 88.621 ppm reported here, but it is not appropriate to conclude that the DPPH-study sample necessarily had greater antioxidant capacity because DPPH and ABTS use different radicals and test conditions. A valid comparison would require testing the same samples and standards under controlled protocols within one study. Imran et al. (2025)

The source also mentions vitamin C as a reference in the discussion, but the quantitative results table available here does not show a curve or IC₅₀ for vitamin C measured alongside the sample. Therefore, this document does not present vitamin C as a verified experimental control. If vitamin C was tested in the original experiment, the raw data should be added to enable a stronger comparison.

Conclusions

The ethyl acetate fraction of paliassa (*Kleinhovia hospita* L.) stem bark exhibited ABTS radical-scavenging activity that increased across the tested concentration range of 20–120 ppm. Mean inhibition rose from 40.374% to 54.536%, and the reported mean IC₅₀ was 88.621 ppm. Phytochemical screening suggested the presence of alkaloids, flavonoids, saponins, and tannins; an additional green-color reaction was provisionally consistent with the steroid indication described in the source procedure, but requires confirmation against the original laboratory record. These findings support radical-scavenging activity under the in-vitro ABTS conditions used, not clinical or therapeutic efficacy. Before submission, the authors must confirm the actual measurement wavelength, the ABTS/potassium persulfate preparation and working absorbance, the phytochemical sample identity, and the final fraction-yield value from primary records.

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Conflicts of Interest

The authors declare no conflict of interest. [AUTHOR: retain the statement that funders had no role only if external funding was received and this description is accurate.]

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