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Antioxidant Activity of Kaik-Kaik (*Uncaria cordata* (Lour.) Merr.) Stems in Various Fraction Levels

Aktivitas Antioksidan Batang Kaik-Kaik (*Uncaria cordata* (Lour.) Merr.) dalam Berbagai Tingkatan Fraksi

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ABSTRACT

Borneo has abundant biodiversity, especially regarding medicinal plants. One of the plants used in traditional medicine by the Dayak tribe in Kalimantan is kaik-kaik (*Uncaria cordata* (Lour.) Merr.), so research is needed to evaluate the activity of this plant. One of the methods used to assess its medicinal properties is through its antioxidant activity. This research aimed to test the antioxidant activity of various fractions derived from *U. cordata* stems. The study began with ethanol extraction followed by fractionation using n-hexane, ethyl acetate, and distilled water. The extracts and fractions obtained were then tested for antioxidant activity using the DPPH radical capture method. The results indicated that the n-hexane fraction, with a yield of 0.69%, contains terpenoids/steroids, alkaloids, and flavonoids; the ethyl acetate fraction, with a yield of 1.453%, contains alkaloids and flavonoids; and the distilled water fraction, with a yield of 4.832%, contains saponins, tannins, alkaloids, phenols, and flavonoids. The n-hexane, ethyl acetate, and distilled water fractions exhibited antioxidant capacity with IC_{50} values of 138.39 ± 0.3777 ppm, 48.51 ± 0.056 ppm, and 36.31 ± 0.111 ppm, respectively. This research concluded that the distilled water fraction was the most active and is classified as a very strong antioxidant.

Keywords: *Uncaria cordata*, DPPH, antioxidants

ABSTRAK

Kalimantan memiliki keanekaragaman hayati yang melimpah khususnya yang dipergunakan dalam hal pengobatan. Salah satu tanaman yang dipergunakan dalam pengobatan oleh suku Dayak yang tinggal di Kalimantan adalah kaik-kaik (*Uncaria cordata* (Lour.) Merr.), sehingga diperlukan penelitian untuk menguji aktivitas tanaman ini. Salah satu pendekatan yang dapat digunakan adalah antioksidan. Tujuan penelitian ini adalah melakukan uji aktivitas antioksidan dari berbagi macam fraksi *U. cordata*. Penelitian ini dimulai dengan ekstraksi etanol dan difraksinasi menggunakan n-heksana, etil asetat, dan akuades. Hasil ekstraksi dan fraksinasi yang diperoleh kemudian diuji antioksidan dengan metode penangkapan radikal DPPH. Hasil dari penelitian ini yaitu fraksi n-heksana dengan rendemen 0.69% mengandung terpenoid/steroid, alkaloid, dan flavonoid; fraksi etil asetat dengan rendemen 1.453% mengandung alkaloid dan flavonoid; fraksi akuades rendemen 4.832 % mengandung saponin, tanin, alkaloid, fenol, dan flavonoid. Fraksi n-heksana, etil asetat dan akuades memiliki kemampuan antioksidan dengan nilai IC_{50} 138.39 ± 0.3777 ppm, 48.51 ± 0.056 ppm, dan 36.31 ± 0.111 ppm. Kesimpulan dari penelitian ini diperoleh fraksi akuades yang paling aktif dan tergolong sebagai antioksidan sangat kuat.

Kata Kunci: *Uncaria cordata*, DPPH, antioksidan

INTRODUCTION

Kalimantan has a variety of natural materials, most of which have medicinal properties. Medicinal plants are a major investment for the welfare of society because herbal medicine is a characteristic of traditional Indonesian medicine. The genus of plants that has been used by the Dayak tribe for generations is *Uncaria sp*., and one of these species that is widely used in medicine is kaik-kaik (*Uncaria cordata* (Lour.) Merr.), with its active content being the flavonoid group (Erwin, 2020).

Flavonoids have been proven to have biological activities such as antivirus, antibacterial, anti-cancer, and stabilising free radicals. *U. cordata* empirically has efficacy against symptoms of dengue virus infection in the wood (Abd Kadir et al., 2013). Infusion of wood parts has anti-inflammatory and antigenotoxic abilities using somatic mutations and recombinant tests of Drosophila melanogaster and is able to inhibit hydrogen peroxidase, with the responsible compound being the phenolic group (Romero-Jiménez et al., 2005). In this plant, α-glucosidase inhibitor compounds were found, namely β-sitosterol, loganin, taxifolin, kaempferol, quercetin, 3,4-dihydroxy-7-methoxycoumarin, scopoletin, 3,4-dihydroxybenzoic acid, and 2-hydroxybenzoic acid (Imran et al., 2010). This plant's leaves have antihypercholesterolemic properties at a concentration of 200 mg/kg body weight (Djohari et al., 2021). The cytotoxic value of *U. cordata* terpenoid wood isolate was 69.18 ppm (Rahmawati & Rusnedy, 2021). Infusion of wood parts has the ability to inhibit *Staphylococcus aureus* by 15.7 mm at a concentration of 100% (Desi et al., 2022). The ethyl acetate fraction in wood has the ability to inhibit the growth of *E. coli* 5.6 mm at a concentration of 300 ppm (Alfionita et al., 2021). Ethanol extract of *U. cordata* has the potential to slow the growth of *S. aureus* and *S. typhi* with a MIC value at a concentration of 4% (Rachmatiah et al., 2020). Endophytic fungi have been successfully isolated from *U. cordata* wood with the code IFED 1 (*Nigrospora sp.),* which has the ability to inhibit the growth of *P. acnes* and *E. coli* (Octaviani et al., 2022). Based on this description, there has been no research on free radical scavengers (antioxidants) in *U. cordata* stem parts with various levels of fractions. Radical compounds are reactive molecules that can affect the macromolecules around them (Fowler & Koffas, 2009). This can cause cell damage that triggers degenerative diseases.

The mechanism of free radical stabilization is that antioxidant compounds donate hydrogen atoms to free radicals, so that these free radicals will be stabilized in the antioxidant structure through the resonance process of π bonds. This reduces the damaging effects of free radicals on other molecules (Mawa et al., 2016). Plants' natural sources of antioxidants play an important role in the body's homeostasis process, such as premature aging (Imran et al., 2010). Free radicals are electrons in a single state in the structure of a compound, so that the compound is unstable and can cause chain reactions (Prakash, 2001). Antioxidant activity tests of various fraction levels are required to group compounds based on their polarity. The n-hexane solvent can attract nonpolar compounds such as terpenoids and alkaloids; the ethyl acetate eluent is included in the semipolar group, which is able to attract active compounds from its group such as flavonoids; while the water solvent can attract polar compounds such as polyphenols (Zuraida et al., 2017). Therefore, this fractionation method serves as the foundation for the research method; aside from this, no research has been conducted on antioxidants found in the stem of *U. cordata* at various levels of fractionation.

MATERIALS AND METHODS

1. Tools and materials

The equipment is an oven (Finco In Ov 50, Taiwan), hair dryer (Maspion, Indonesia), UV-Vis spectrophotometry (Perkin Elmer, Singapore), TLC plate (Merck, Germany), analytical balance (Ohaus PAJ1003, USA), and water bath (Menmert, Germany).

The main ingredients are *U. cordata* stem, 70% ethanol, n-hexane, DPPH (Sigma-Aldrich, St. Louis, United States), quercetin (Sigma-Aldrich, St. Louis, United States), methanol p.a. (Merck, Germany), distilled water, butanol, acetic acid, Dragendorf reagent, Liebermann-Burchad reagent, $AlCl_3$ reagent, $FeCl_3$ reagent, and H_2SO_4 reagent.

2. Extraction and fractionation

This study started by collecting *U. cordata* stems, which were then oven-dried at 50 ^{0}C for 3x24 hours. The obtained simplicia was then powdered using a blender and sieved through 40 mesh. A sample of 100 g was then macerated using 70% ethanol for three days and occasionally stirred, and re-maceration was carried out twice. The maceration results obtained were then filtered using filter paper; the liquid extract was then evaporated using a water bath at 55 ˚C until a thick extract was obtained.

The resulting 70% ethanol-thick extract was then dissolved in 100 mL of distilled water and put into a separating funnel using n-hexane solvent (1:1). This fractionation was replicated twice. The distilled water fraction was then added with ethyl acetate solvent (1:1).

Both solvents will separate. The results of each fractionation were concentrated to obtain a thick fraction. The n-hexane, ethyl acetate, and distilled water fractions were then identified for chemical compounds using the tube method (Panca et al., 2022).

3. Antioxidant test

Samples (n-hexane fraction, ethyl acetate, and aquadest) of 10 mg were given methanol p.a. up to 10 ml, then made at various concentrations. A total of 1 ml of DPPH 0.1 mM solution in 4 ml, then incubated for operational time, then read the absorbance of the wavelength that has been set with a spectrophotometer (Alyidrus et al., 2021). This antioxidant test research was conducted three times in replication.

4. Data analysis

Quantitative data includes antioxidant activity testing based on the IC_{50} value. The value is obtained from linear regression between test concentration and inhibition percentage. The smaller the IC_{50} value, thehigher the activity using Microsoft Excel.

$$\%\ antioxidant\ activity = \frac{blank\ absorbance - sample\ absorbance}{blank\ absorbance} X\ 100\%$$

RESULTS AND DISCUSSION

1. Determination and yield of extract

The determination yielded the scientific name *Uncaria cordata* (Lour.) Merr., a synonym for Uruparia multiflora. K.Schum. & Lauterb identified Uruparia nemorosa (Korth.) Kuntze, along with Uruparia pedicellata (Roxb.) Kuntze, and assigned the number 014/LLB.LABDASAR/I/2022.

The process of making simplicia begins with drying the stem simplicia in an oven at a temperature of 50 °C. The purpose of drying is to reduce the water content so that it can stop the enzymatic reaction. This helps shield the sample from fungus overgrowth. The sample is ground with a blender to facilitate the extraction process, where the smaller the particle size, the greater the contact between the sample and the solvent. The sieve used uses a size of 40 mesh (Scherphof & Fahr, 2018). After extraction, fractionation was carried out with various levels of polarity. Additionally, the solvent's polarity difference will separate several chemical compounds in the sample into simpler ones, facilitating their identification. The n-hexane, ethyl acetate, and aquadest fractions yielded 0.690 g, 1.453 g, and 4.832 g, respectively.

Table 1 presents data related to the weight of the simplicia. The next step is to test the chemical compounds in each fraction. The n-hexane fraction contains flavonoids, steroids, and alkaloids. The ethyl acetate fraction contains flavonoids, alkaloids, and phenols. The chemical compounds in the aquades fraction contain flavonoids, steroids, saponins, tannins, alkaloids, and phenols. **Table 2**: This content is slightly different from the methanol extract of the wood part, which contains flavonoids, phenols, and tannins. (Ahmad et al., 2011). It is impossible to distinguish the groups of compounds in the tube using the qualitative analysis method, which does not use a comparator and instead relies on changes in color reactions. Therefore, alternative methods of analysis are required.

Tabel 1. Yield of *U. cordata* stem fraction

Sample	Yield (%)
n-hexane	0.690
Ethyl acetate	1.453
Aquades	4.832

Tabel 2. Phytochemical test results of *U. cordata* stem extract

Compound	n-hexane fraction	Ethyl acetate fraction	Aquades fraction
Flavonoids	+	+	+
Terpenoids/steroids	+	-	-
Saponins	-	-	+
Tannins	-	-	+
Alkaloids	+	+	+
Phenols	-	+	+

2. Antioxidant test

We use the DPPH method to test antioxidant activity because it is simple, fast, easy, and requires a small amount of sample in a short time. Other advantages include accuracy and practicality when used. This method's principle involves a color change from purple to yellow, which corresponds to an increase in the concentration of antioxidants used. This change is directly proportional to the increase in electron-pairing radicals. In other words, this antioxidant works by changing the colour of something because it stops the production of free radicals. This happens when DPPH reacts with the sample compound molecules' release of hydrogen. This color change leads to a shift in absorbance from the maximum wavelength of DPPH, which enables us to calculate the known percentage of inhibition (Molyneux, 2004). Determine the maximum DPPH wavelength by determining the concentration of the compound that gives maximum absorbance. Measurements at the maximum wavelength will provide linear results, high instrument sensitivity, and reduce

measurement errors (Sadeli, 2016). The absorbance measurement of a 0.1 mM DPPH solution in the range of 450–550 nm determines the DPPH wavelength. The results were obtained at a wavelength of 516 nm; this value is close to other studies, specifically 517 nm (Oroian & Escriche, 2015). The determination of operational time with DPPH is when the compound containing antioxidants reaches a stable point.

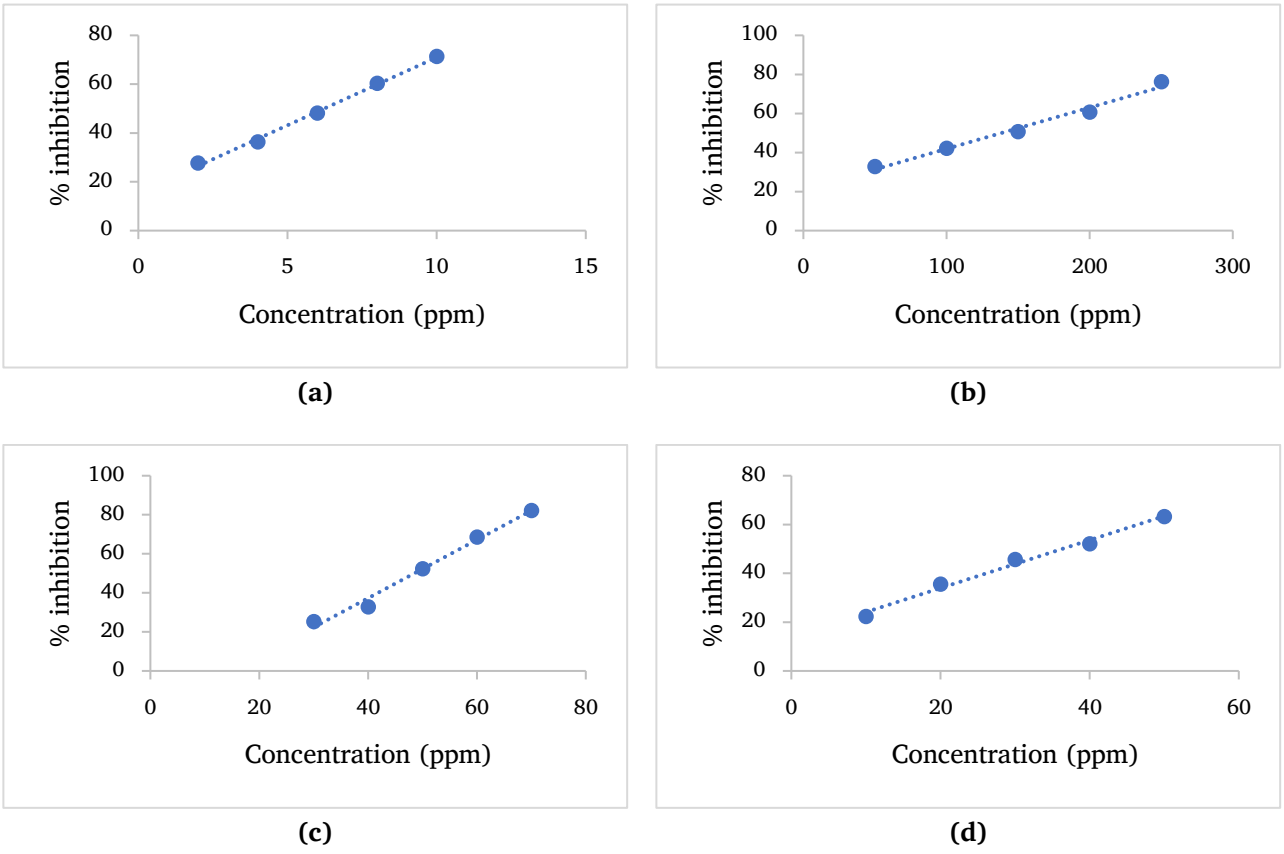

Figure 1. The graph displays the average percentage of inhibition for various compound concentrations and fractions, with three replications. The compounds include (**a**) quercetin, (**b**) n-hexane fraction, (**c**) ethyl acetate fraction, and (**d**) aquadest fraction.

The process of determining the operational time begins with absorbance measurement. A 4 ppm sample was selected containing a comparison solution of quercetin and DPPH, which had been reacted starting from the 5th minute to the 60th minute with a UV-Vis spectrophotometry device until a stable absorbance was obtained. The results of determining the operational time were obtained at 10–25 minutes indicating the stability of absorbance in accordance with the quercetin research conducted by Jafar (Dolatabadi et al., 2014). The study used quercetin as validation to determine the truth of the test that had been carried out and as a comparison or positive control because it was able to show radical scavenging with an IC_{50} of 6.23 ± 0.007 ppm with the equation Y = 5.5635X + 15.339 and an R2 value of 0.9971. According to these results, **Figure 1a** states that quercetin has very strong antioxidant activity. The next stage is sample testing. The results obtained in the n-hexane, ethyl acetate, and aquadest fractions were 138.39 ± 0.377 ppm, 48.51 ± 0.056 ppm, and 36.31 ± 0.111 ppm, respectively. The equation from the n-hexane fraction yields this value: Y = 0.2112X + 20.772, with an R2 value of 0.9842 (**Figure 1b)**. Ethyl acetate fraction equation Y = 1.4943X – 22.487 and R2: 0.9863 (**Figure 1c)**. Aquadest fraction The obtained equation is Y = 0.9836X + 14.276, with an R2 value of 0.9881 (**Figure 1d**). The R2 value obtained is above 0.98, so there is a close relationship between concentration and inhibition (BPOM RI, 2013). The n-hexane fraction is in the medium category for antioxidants, while the ethyl acetate and aquadest fractions are in the very strong category. The n-hexane fraction obtained an RSD of 0.272 from the average measurement of antioxidants; the ethyl acetate fraction obtained an RSD of 0.115, while the ethyl acetate fraction obtained an RSD of 0.306. The values obtained from the three RSDs were below 5%, indicating that the data deviation was low and met the requirements for good data precision *(Ramos* et al., 2017).

The antioxidant value obtained in this study varied among different fractions. This is because the compounds from the stems were collected based on

polarity. The IC_{50} value from the methanol extract of the leaves, which was 200 ppm, and the IC_{50} value from the wood portion, which was 80 ppm, appear to be different (Ahmad et al., 2011). In other studies, Uncaria has different activities, namely the stem part of *U. lucida*, *U. longiflora* v.p., and U. colophylla, each of which is 65 ppm, 10 ppm, and 20 ppm, with the extraction solvent being methanol (Ahmad et al., 2011), this is due to the extraction of the flavonoids in the form of glycosides, which are responsible for the antioxidant effect *(Irianti* et al., 2016). According to research on the activity of *U. cordata* leaves and roots, it has antioxidant activity in the ethanol fraction of 49.22 ppm, the n-hexane fraction of 108.22 ppm, and the ethyl acetate fraction of 13.30 ppm (Silvia et al., 2022). Root infusion of Uncaria Gambir Roxb, synonym of *U. cordata*, has an antioxidant capacity of 71.77 ppm (Salsabila et al., 2023), It provides information to the public regarding which parts of the plant will be used, as well as information for research when continuing to isolate active compounds, with diverse antioxidant activities.

CONCLUSION

The antioxidant test of *U. cordata* stems with different fractions showed that the n-hexane fraction has a moderate effect (IC_{50} = 138.39 ± 0.377), the ethyl acetate fraction has an IC_{50} = 48.51 ± 0.056, and the aquadest fraction has an IC_{50} = 36.31 ± 0.1111, which means it is very strong. The aquadest fraction has the strongest activity, and the best use in the community is through boiling.

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