Jurnal Pertanian Terapan of JURNAL ROCE (AGROSCIENCE)
https://jurnal.rocewisdomaceh.com/index.php/roce e-ISSN : 3032-4505
https://doi.org/10.71275/roce.v2i1.128

ANTIOXIDANT OF n-HEXANE EXTRACT OF *Uncaria gambir* Roxb FROM PONTIANAK

ANTIOKSIDAN EKSTRAK n-HEXANE *Uncaria Gambir* Roxb DARI PONTIANAK

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Abstract

The n-hexane extract of dried UGR leaves was obtained with a yield of 8.0 grams (16%). Phytochemical screening revealed the presence of secondary metabolites, including phenolics, steroids, and terpenes. The antioxidant activity was evaluated using the DPPH assay, yielding an IC50 value of 53.654 ppm, which classified the extract as strong, falling within the 50–100 ppm range. However, n-hexane extracts generally exhibit weaker antioxidant activity due to the nonpolar nature of the solvent, which limits the extraction of polar antioxidant compounds such as polyphenols and flavonoids. Consequently, n-hexane extracts contain higher amounts of lipids, terpenes, and other nonpolar compounds, which are less effective at scavenging DPPH radicals compared to polar extracts like ethanol or methanol.

Keywords: Uncaria gambir Roxb, n-hexane, antioxidant, phytochemicals

Abstrak

Ekstrak n-heksana dari daun UGR kering diperoleh dengan rendemen sebesar 8,0 gram (16%). Skrining fitokimia menunjukkan adanya metabolit sekunder, termasuk senyawa fenolik, steroid, dan terpen. Aktivitas antioksidan dievaluasi menggunakan uji DPPH, menghasilkan nilai IC50 sebesar 53,654 ppm, sehingga ekstrak ini dikategorikan kuat dalam rentang 50–100 ppm. Namun, secara umum ekstrak n-heksana menunjukkan aktivitas antioksidan yang lebih lemah karena sifat pelarut yang non-polar, yang membatasi ekstraksi senyawa antioksidan polar seperti polifenol dan flavonoid. Akibatnya, ekstrak n-heksana mengandung lebih banyak lipid, terpen, dan senyawa non-polar lainnya, yang kurang efektif dalam menangkap radikal DPPH dibandingkan ekstrak polar seperti etanol atau metanol.

Kata Kunci : Uncaria gambir Roxb, n-heksan, antioksidan, fitokimia

INTRODUCTION

N-Hexane is commonly used as a solvent for plant extraction. For example, the n-hexane extract of *Blumea balsamifera* L. leaves and its fractions were evaluated for antioxidant and antiproliferative activities. The fractionation process produced one n-hexane extract and ten distinct fractions. Results showed that all samples exhibited weak antioxidant activity (IC_{50}

> 100 ppm), with fraction 8 showing the highest activity (IC_{50} = 113.716 ppm) (Ginting *et al*., 2022).

N-Hexane was also used to obtain a fraction from *Azanza garckeana* leaf extract to examine its phytochemical content, bioactive compounds, and antioxidant potential. The screening revealed that the n-hexane fraction contained relatively high levels of tannins, moderate amounts of saponins, and low steroid content. Antioxidant tests revealed that the crude extract could scavenge DPPH radicals, with activity increasing as the concentration decreased, although it remained weaker than that of ascorbic acid. GC-FID analysis identified 19 compounds within the n-hexane fraction, confirming that *A. garckeana* leaves contain bioactive compounds beneficial to health and serve as a potential antioxidant source (Nkwocha *et al*., 2024).

Furthermore, n-hexane was used to fractionate the crude extract of *Rhynchosia nulubilis* cultured with *Ganoderma lucidum* mycelium (RNGM). The n-hexane fraction was analyzed for anticancer and anti-inflammatory activities, as well as its content of polyphenols, flavonoids, isoflavones, and β-glucans. The results indicated that the fraction contained 0.85% β-glucans, demonstrating its potential as a natural source for nutritional and pharmaceutical applications. In this study, n-hexane extraction was performed on *Uncaria gambir* Roxb (UGR) leaves to evaluate their antioxidant and antibacterial activities. UGR was selected based on reports indicating that its stems exhibit diverse biological activities, including antioxidant, antibacterial, anthelmintic, anticancer, antifungal, anti-inflammatory, hypoglycemic, antihyperuricemic, lipid peroxidation inhibitory, blood lipid-regulating, and antifungal effects (Munggari *et al*., 2022; Pramanik *et al*., 2023; Hidayati & Rahmatulloh, 2022).

RESEARCH METHODS

UGR Leaf Extraction

Dried UGR leaves were blended and weighed to 50 grams. They were then soaked in n-hexane for three days, filtered, and concentrated using a rotary evaporator to obtain a dry extract.

Phytochemical Screening of n-Hexane Extract from UGR Leaves

Alkaloid Test: A 2 ml sample solution was prepared and heated in a porcelain dish until only a dry extract remained. The residue was then dissolved in 5 mL of 2N HCl and divided into four test tubes. Diluted acid served as a blank; three drops of Dragendorff reagent were added to the second tube, three drops of Mayer reagent to the third tube, and three drops of Wagner reagent to the fourth tube. The appearance of a red precipitate in tube two, yellow in tube three, and brown or red in tube four indicated the presence of alkaloids.

Phenolic Test: The sample solution was split into two test tubes, with tube A serving as the blank and tube B reacting with a 10% $FeCl_3$ solution. A change to bluish-black confirmed the presence of phenolic compounds. Flavonoid Test: 2 ml of the sample was added to a test tube, followed by three drops of 10% NaOH. The solution was then divided into two tubes, with tube A as the blank. A yellow color in tube B indicated flavonoids, while a bluish-black color indicated phenols.

Terpene Test: 2 ml of the sample was evaporated in a porcelain dish. After drying, Ciulei reagent (0.5 mL of chloroform, 0.5 mL of acetic anhydride, and 2 mL of concentrated sulfuric acid) was added. The emergence of a brown color indicated the presence of terpenoids.

Saponin Test: 2 ml of the sample was placed in a test tube, 10 ml of distilled water was added, and the mixture was homogenized. The formation of foam indicated saponins. All tests were carried out in duplicate (Iskandar & Warsidah, 2020).

Antioxidant Activity Determination Using the DPPH Radical Scavenging Method:

The stock solution was prepared by dissolving 10 mL of UGR extract in 50 mL of ethanol and diluted as needed. The DPPH stock solution was prepared by dissolving 5 mg of DPPH in 100 mL of methanol and homogenizing it using a vortex. For the assay, 2 mL of each sample was mixed with 2 mL of DPPH solution. The mixtures were incubated at 27°C for 30 minutes, until a color change occurred, with duplicate samples for each treatment. Color changes were measured using a spectrophotometer at a wavelength of 517 nm. All measurements were performed twice (Baliyan *et al*., 2022).

RESULTS AND DISCUSSION

Figure 1. Results of Phytochemical Screening Test of UGR Leaf n-Hexane Extract

Table 1. Phytochemical Screening Results of UGR Leaf n-Hexane Extract

Secondary Metabolites	Results
Flavonoids	-
Phenolic	+
Steroids/terpenes	++
Saponins	-
Alkaloids	
Wagner	-
Meyer	-
Dragendroff	-

Table 2. Antioxidant Test Results of UGR Leaf n-Hexane Extract

Concentration (ppm)	Blank	Absorbance (Duplo)	% Inhibition	Means (%)	IC_{50}
5	0.791	0.666	15.803	18.584	53.654
		0.622	21.365		
10		0.61	22.882	22.566	
		0.615	22.250		
15		0.618	21.871	23.009	
		0.600	24.147		
20		0.603	23.767	24.968	
		0.584	26.169		
25		0.565	28.571	33.818	
		0.482	39.064		

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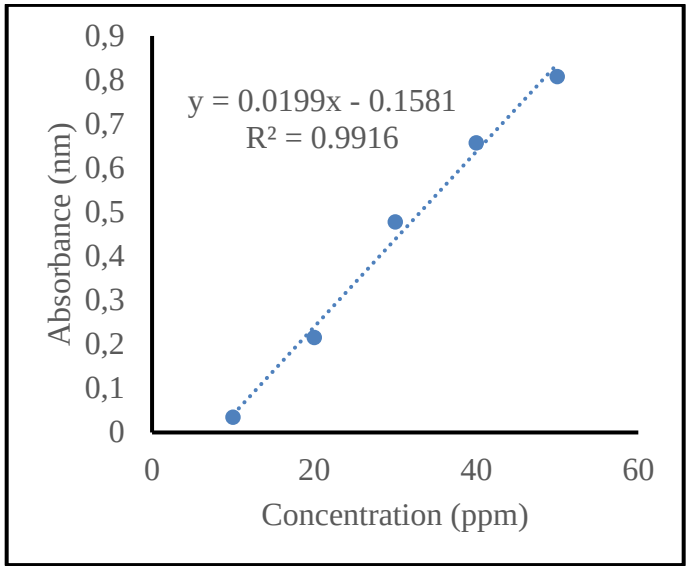

Figure 2. Standard Curve for Determination of IC50 Antioxidant of UGR Leaf n-Hexane Extract

The n-hexane extract of dried UGR leaves yielded 8.0 grams, equivalent to a 16% extraction yield. Phytochemical analysis confirmed the presence of secondary metabolites, including phenolics, steroids, and terpenes. The antioxidant activity, as measured by the DPPH assay, is presented in Table 2 and Figure 1. From the linear regression analysis in Figure 1, the IC50 value of the antioxidant activity of the n-hexane extract of UGR leaves was determined to be 53.654 ppm. Based on IC50 classification, this extract is considered strong, falling within the 50–100 ppm range (Maghfirah *et al*., 2025). Its antioxidant potency is higher compared to the n-hexane extract of *Blumea balsamifera* L. leaves (IC50 = 281.707 ppm) (Ginting *et al*., 2022) and the n-hexane extract of tobacco leaves (IC_{50} = 426.042 ppm) (Patricia *et al*., 2025). N-hexane extracts tend to have a weak antioxidant activity, as indicated by their IC50 values, due to several factors. Solubility of active compounds: The main antioxidant compounds, such as polyphenols and flavonoids, are generally polar. Since n-hexane is a nonpolar solvent, it can only extract a small amount of these polar compounds, resulting in low concentrations of antioxidants (Fioroni *et al*., 2023). N-hexane extracts typically contain more lipids, terpenes, and other nonpolar compounds, which are chemically less effective at scavenging DPPH radicals than polar compounds such as flavonoids or phenolic acids (Nkwocha *et al*., 2024). Leaves or plant materials that contain little non-polar antioxidant compounds, then the n-hexane extract will naturally show weaker antioxidant activity than polar extracts such as ethanol or methanol (Akullo *et al*., 2023).

CONCLUSION

Based on the results, the n-hexane extract of UGR leaves yielded 16% and contained secondary metabolites, including phenolics, steroids, and terpenes. Antioxidant activity, as tested using the DPPH method, revealed an IC50 value of 53.654 ppm, which is classified as strong (50–100 ppm). This activity was higher compared to the n-hexane extract of *Blumea balsamifera* leaves and tobacco leaves. Although n-hexane extracts are generally weak in antioxidant activity due to their nonpolar nature, which limits the extraction of phenolic or flavonoid compounds that are more polar, UGR leaves were proven to produce vigorous

antioxidant activity, indicating the presence of nonpolar compounds with significant free radical scavenging potential.

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