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RESEARCH ARTICLE

ANTIOXIDANT POTENTIAL OF METHANOLIC ROOT EXTRACT OF *Uncaria gambir* Roxb.: EVIDENCE FROM A DPPH RADICAL SCAVENGING ASSAY

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ABSTRAK

Akar gambir (*Uncaria gambir* Roxb.) merupakan bagian tanaman yang masih kurang dimanfaatkan meskipun berpotensi mengandung senyawa dengan aktivitas antioksidan. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan ekstrak metanol akar gambir menggunakan metode penangkapan radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH) serta melakukan skrining fitokimia awal. Akar gambir dikeringkan, diekstraksi dengan metode maserasi menggunakan metanol 70%, dan diperoleh rendemen ekstrak sebesar 10,64%. Skrining fitokimia menunjukkan adanya fenolik, alkaloid, saponin, terpenoid, dan flavonoid, sedangkan steroid tidak terdeteksi. Aktivitas antioksidan diuji pada konsentrasi akhir 6,25; 3,125; 1,562; 0,781; dan 0,39 µg/mL. Ekstrak menunjukkan aktivitas penangkapan radikal DPPH yang meningkat seiring peningkatan konsentrasi, dengan nilai inhibisi rata-rata berkisar antara 4,40 ± 0,17% hingga 26,27 ± 0,14%. Analisis regresi linear dari tiga replikasi menghasilkan nilai IC_{50} masing-masing sebesar 12,58; 12,53; dan 12,60 µg/mL, dengan rerata IC_{50} sebesar 12,57 ± 0,04 µg/mL, sedangkan asam galat menunjukkan nilai IC_{50} yang lebih rendah, yaitu 3,06 ± 0,05 µg/mL. Hasil ini memberikan bukti awal bahwa akar gambir memiliki potensi antioksidan dan dapat menjadi dasar untuk karakterisasi fitokimia lebih lanjut.

Kata kunci: Akar, Antioksidan, DPPH, IC_{50}, *Uncaria gambir* Roxb.

ABSTRACT

The root of *Uncaria gambir* Roxb. remains relatively underutilized despite its potential as a source of antioxidant constituents. This study aimed to evaluate the antioxidant activity of the methanolic root extract of *U. gambir* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and to perform preliminary phytochemical screening. Dried roots were extracted by maceration with 70% methanol, yielding 10.64% extract. Phytochemical screening showed the presence of phenolics, alkaloids, saponins, terpenoids, and flavonoids, whereas steroids were not detected. Antioxidant activity was assessed at final concentrations of 6.25, 3.125, 1.562, 0.781, and 0.39 µg/mL. The extract exhibited concentration-dependent DPPH radical scavenging activity, with mean inhibition values ranging from 4.40 ± 0.17% to 26.27 ± 0.14%. Linear regression analysis from three replicates gave IC_{50} values of 12.58, 12.53, and 12.60 µg/mL, with a mean IC_{50} of 12.57 ± 0.04 µg/mL, whereas gallic acid showed a lower IC_{50} of 3.06 ± 0.05 µg/mL. These findings provide preliminary evidence that *U. gambir* roots have antioxidant potential and support further phytochemical characterization to identify the bioactive compounds responsible for the observed activity.

Keywords: Antioxidant, DPPH, IC_{50}, Root extract, *Uncaria gambir* Roxb

INTRODUCTION

Free radicals play a crucial role in the pathogenesis of various degenerative diseases, including diabetes mellitus, cardiovascular disorders, and premature aging (Scarpa ES *et al.*, 2024). Antioxidant compounds function by neutralizing free radicals through electron or hydrogen atom donation mechanisms, thereby reducing oxidative damage within biological systems (Parcheta *et al.*, 2021). Consequently, the exploration of natural antioxidant sources from plants continues to receive considerable attention in pharmaceutical and natural product research.

Uncaria gambir Roxb., commonly known as gambir, is recognized as a rich source of phenolic compounds, particularly catechins and proanthocyanidin derivatives, which contribute to its notable antioxidant activity (Hidayati and Rahmatulloh, 2022). Most previous studies on gambir have focused primarily on the leaves or commercially processed gambir products, while other plant parts remain relatively underexplored (Ningsih and Rahayuningsih,

2019). One such part is the root, which plays a vital role in nutrient uptake and soil interaction and may accumulate secondary metabolites as part of the plant's adaptive and defense mechanisms.

Several reports indicate that woody plant roots may contain significant levels of phenolic compounds and tannins that contribute to antioxidant activity (Benramdane *et al.*, 2025). However, scientific data regarding the antioxidant activity of gambir roots remain limited, particularly studies employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Variations in plant parts investigated, extraction solvents used, and analytical approaches have further limited direct comparisons across studies. Therefore, an exploratory preliminary investigation is necessary to provide baseline data on the biological potential of gambir roots.

Methanol is widely recognized as an effective solvent for extracting phenolic compounds and tannins and is commonly employed in *in vitro* antioxidant screening studies (Altemimi *et al.*, 2017). In the DPPH assay, antioxidant activity is typically evaluated across multiple concentration levels to establish a linear relationship between concentration and percentage inhibition (Apea-Bah FB *et al.*, 2009). Previous studies have reported antioxidant activity in *U. gambir* from different plant parts and extraction systems. Salsabilla et al. (2023) reported DPPH radical scavenging activity of infused bajakah kalalawit roots (*U. gambir*), while Aris et al. (2025) evaluated the ethanol extract of *U. gambir* roots using the FRAP assay (Salsabila, Febriyanti and Amananti, 2023; Aris, Baits and Suhaenah, 2025). Indriyah et al. (2023) also reported antioxidant activity in the stem extract and fractions of bajakah kalalawit using the FRAP method (Indriyah, Permatasari and Pratama, 2023). Although previous studies have evaluated antioxidant activity in U. gambir roots using different extraction systems or antioxidant assays, studies specifically examining methanolic root extracts of *U. gambir* using the DPPH radical scavenging assay remain limited. Accordingly, this study was conducted as a preliminary investigation to evaluate the antioxidant potential of the methanolic root extract of *U. gambir* using the DPPH radical scavenging method, with gallic acid as a reference antioxidant.

RESEARCH METHODS

Equipment

The equipment used in this study included a mechanical grinder for pulverizing the dried plant material, an analytical balance (Shimadzu, Japan) for accurate weighing, and a rotary evaporator (Buchi, Switzerland) operated under reduced pressure for solvent removal. A UV–Vis spectrophotometer (Shimadzu, Japan) set at 517 nm was employed to measure absorbance during the DPPH assay. Standard laboratory glassware from Pyrex, including beakers, volumetric flasks, pipettes, and cuvettes, was used throughout the experimental procedures. Whatman No. 1 filter paper was utilized for filtration, and incubation at room temperature was conducted under controlled laboratory conditions. All instruments were calibrated prior to use to ensure the accuracy and reliability of the measurements.

Materials

Plant Material

Fresh and healthy roots of *Uncaria gambir* Roxb. (approximately 1 kg) were collected from the Medicinal Plant Garden (KTOF), Laboratory of Sumatra Biota, Universitas Andalas. Plant identification was conducted based on taxonomic characteristics, and a voucher specimen was deposited at the Herbarium of Universitas Andalas for reference under voucher number 343/K-ID/ANDA/2019 for future reference.

The roots were washed with distilled water to remove soil and surface contaminants, air-dried under shade at approximately 20 °C for three days, and then ground into powder (Anggraini T *et al.*, 2011). The powdered material was stored in tightly sealed containers protected from light until extraction.

Chemicals and Reagents

The chemicals and reagents used in this study included 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, St. Louis, MO, USA), gallic acid (Sigma-Aldrich, St. Louis, MO, USA) as the reference antioxidant standard, and methanol p.a. (Merck, Darmstadt, Germany) as the solvent for extraction, dilution, and DPPH reagent preparation. All reagents were of analytical grade and used without further purification.

Procedures

Preparation of Methanolic Extract

Powdered gambir roots weighing 10.21 g were extracted by maceration using 100 mL of 70% methanol at ambient laboratory temperature (approximately 28–30 °C) in a well-ventilated room, corresponding to a sample-to-solvent ratio of approximately 1:10 w/v. The maceration was carried out for nine days, with the solvent replaced every three days to optimize extraction efficiency (Dias *et al*. 2021). After maceration, the mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure

using a rotary evaporator to obtain a thick extract free of solvent. The extraction yield was 10.64%. The concentrated extract was stored in an airtight container protected from light at 4 °C until further analysis.

Phytochemical Screening

The concentrated semi-solid extract (50 mg) was dissolved in methanol and subjected to qualitative phytochemical screening for phenolics, alkaloids, saponins, steroids, terpenoids, and flavonoids according to the methods described by Harborne (Harborne 1973).

Determination of Antioxidant Activity (DPPH Assay)

Antioxidant activity was evaluated using the DPPH radical scavenging method adapted from a previous report with slight modifications (Abramovič *et al.*, 2017). The DPPH reagent was prepared by dissolving 5 mg of DPPH in 200 mL of analytical-grade methanol to obtain a 0.025 mg/mL DPPH solution. The methanolic root extract of *Uncaria gambir* Roxb. was initially prepared as a stock solution by dissolving 10 mg of extract in 10 mL of analytical-grade methanol to obtain a concentration of 1,000 µg/mL. The stock solution was then serially diluted to obtain test solutions of 125, 62.5, 31.24, 15.62, and 7.81 µg/mL. For each assay, 200 µL of each test solution was mixed with 3.8 mL of DPPH solution in a cuvette, corresponding to a sample-to-DPPH solution volume ratio of 1:19. This produced final extract concentrations in the reaction mixture of 6.25, 3.125, 1.562, 0.781, and 0.39 µg/mL, respectively. Methanol was used as the blank by replacing the sample solution with 200 µL of methanol. Gallic acid was used as the positive control and was prepared and tested under the same assay conditions. The absorbance was measured at 517 nm after 30 minutes of incubation at room temperature in the dark using a UV–Vis spectrophotometer. Each concentration was tested in triplicate.

Determination of IC_{50}

The percentage of DPPH inhibition was calculated using the following equation:

$$\%\ Inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}} x\ 100$$

where A_{blank} is the absorbance of the DPPH solution with methanol, and A_{sample} is the absorbance of the DPPH solution containing the extract or gallic acid. The IC_{50} value was obtained from the linear regression equation between sample concentration and percentage inhibition. The general form of the regression equation was expressed as y = ax + b, where y represents the percentage of inhibition, x represents the sample concentration, a is the slope, and b is the intercept. The IC_{50} value was calculated by substituting y = 50 into the equation and solving for x. A lower IC_{50} value indicates higher antioxidant activity.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 22. The IC_{50} values obtained from three replicate assays for each group were used for statistical comparison. Data normality was assessed using the Shapiro–Wilk test, while homogeneity of variance was evaluated using Levene's test. Since the data were normally distributed and showed homogeneous variance, comparisons between the methanolic root extract and gallic acid were performed using an independent samples t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening of the methanolic root extract of *Uncaria gambir* Roxb. showed positive results for phenolics, alkaloids, saponins, terpenoids, and flavonoids, whereas steroids were not detected (Table 1). The presence of phenolics and flavonoids may contribute to the antioxidant activity of the extract through hydrogen atom donation, electron transfer, and radical stabilization. This finding is consistent with previous studies on *U. gambir* roots. Salsabilla et al. (2023) reported the presence of alkaloids, saponins, flavonoids, tannins, and terpenoids in bajakah kalalawit roots (*U. gambir*), while Aris et al. (2025) confirmed phenolics, alkaloids, flavonoids, and tannins in the ethanol root extract (Salsabila, Febriyanti and Amananti, 2023; Aris, Baits and Suhaenah, 2025). Therefore, the phytochemical profile observed in the present study provides preliminary support for the antioxidant potential of *U. gambir* roots, although further quantitative phytochemical analysis is needed to identify the major compounds contributing to the observed activity.

The methanolic root extract of *Uncaria gambir* Roxb. exhibited concentration-dependent DPPH radical scavenging activity. The percentage of DPPH inhibition increased with increasing final extract concentration. At final concentrations of 0.39, 0.781, 1.562, 3.125, and 6.25 µg/mL, the extract showed mean DPPH inhibition values of 4.40 ± 0.17%, 5.78 ± 0.04%, 8.49 ± 0.25%, 14.80 ± 0.04%, and 26.27 ± 0.14%, respectively.

Table 1. Qualitative Phytochemical Screening of the Methanolic Root Extract of *Uncaria gambir* Roxb.

Samples	Phenolics	Alkaloids	Saponins	Steroids	Terpenoids	Flavonoids
Roots	+	+	+	-	+	+

Note: (+) indicates the presence of phytochemical constituents; (−) indicates not detected.

The concentration-dependent DPPH inhibition values of the methanolic root extract are presented in Table 2. Linear regression analysis between final extract concentration and percentage inhibition produced the equations y = 3.7463x + 2.859, y = 3.7703x + 2.7758, and y = 3.7459x + 2.8075 for the three replicates, respectively, with R^2 values of 0.999, 0.9993, and 0.9994. In these equations, y represents the percentage inhibition and x represents the extract concentration. Based on these equations, the IC_{50} values were 12.58, 12.53, and 12.60 µg/mL, with a mean IC_{50} value of 12.57 ± 0.04 µg/mL. This value suggests that the methanolic root extract of *U. gambir* has antioxidant potential in the DPPH radical scavenging assay.

Table 2. DPPH radical scavenging activity of the methanolic root extract of *Uncaria gambir* Roxb.

Sample	Final concentration (µg/mL)	DPPH inhibition (%)
Methanolic root extract	0.39	4.40 ± 0.17
Methanolic root extract	0.781	5.78 ± 0.04
Methanolic root extract	1.562	8.49 ± 0.25
Methanolic root extract	3.125	14.80 ± 0.04
Methanolic root extract	6.25	26.27 ± 0.14
Gallic acid	0.39	13.08 ± 2.49
Gallic acid	0.781	18.32 ± 2.26
Gallic acid	1.562	32.86 ± 1.27
Gallic acid	3.125	53.70 ± 0.81
Gallic acid	6.25	90.02 ± 0.48

Data are presented as mean ± SD from three replications.

For comparison, gallic acid used as a positive control exhibited a lower IC_{50} value of 3.06 ± 0.05 µg/mL, reflecting stronger antioxidant activity. A comparison of IC_{50} values between the methanolic root extract of *Uncaria gambir* and gallic acid in the DPPH assay is presented in Table 3. This finding is consistent with the known high antioxidant capacity of gallic acid, a pure phenolic compound capable of efficient hydrogen atom donation and radical stabilization through aromatic resonance (Abramovič *et al.*, 2017). The observed difference between the extract and gallic acid is reasonable, considering that plant extracts represent complex mixtures of secondary metabolites with variable contributions to overall antioxidant capacity.

Table 3. Comparison of IC_{50} Values of Methanolic Root Extract of *Uncaria gambir* and Gallic Acid in the DPPH Assay

Sample	IC_{50} (µg/mL) (mean ± SD)
***Uncaria gambir* roots**	12.57 ± 0.04
Gallic Acid	3.06 ± 0.05

Data are presented as mean ± SD from three replications.

The IC_{50} values used for statistical analysis were obtained from three replicate assays for each group. The data were normally distributed based on the Shapiro–Wilk test for both the methanolic root extract (p = 0.337) and gallic acid (p = 0.312). Levene's test indicated homogeneity of variances (F = 1.194, p = 0.336). Therefore, the independent samples t-test was interpreted using the assumption of equal variances. The t-test showed a significant difference in IC_{50} values between the methanolic root extract and gallic acid groups, t(4) = 281.772, p < 0.001.Previous studies have reported antioxidant activity in *U. gambir* from different plant parts and extraction systems. Salsabilla et al. (2023) reported DPPH radical scavenging activity of infused bajakah kalalawit roots (*U. gambir*), with an IC_{50} value of 71.77 µg/mL. Aris et al. (2025) reported antioxidant activity of the ethanol extract of *U. gambir* roots using the FRAP assay, with a value of 193.403 mg QE/g extract. In addition, Indriyah et al. (2023) reported antioxidant activity in the stem extract and fractions of

bajakah kalalawit, with FRAP IC_{50} values ranging from 12.572 to 75.319 µg/mL. Compared with these reports, the present study provides additional preliminary evidence that *U. gambir* roots may contain antioxidant constituents, particularly when extracted using methanol and evaluated using the DPPH assay. Differences in antioxidant activity among studies may be influenced by the plant part used, extraction solvent, assay method, and phytochemical composition. However, antioxidant values obtained from DPPH and FRAP assays should be compared cautiously because the assays are based on different reaction mechanisms.

The antioxidant activity observed in this study may be associated with the presence of phenolics and flavonoids detected in the phytochemical screening. Phenolic compounds and condensed tannins in *U. gambir* have been reported to contribute to antioxidant activity through electron transfer or hydrogen atom donation mechanisms, which are consistent with the principle of the DPPH assay (Apea-Bah FB *et al.*, 2009; Kassim MJ *et al.*, 2011). The use of methanol as a polar extraction solvent may also support the extraction of phenolic and tannin-related compounds, as polar solvents are commonly used for recovering antioxidant constituents from plant materials (Altemimi *et al.*, 2017). Nevertheless, the present study remains preliminary because it was limited to a methanolic extract and a single *in vitro* antioxidant assay. Further studies using a broader concentration range, quantitative phytochemical analysis, and complementary antioxidant assays are required to confirm the antioxidant potency and identify the major bioactive compounds responsible for the observed activity.

CONCLUSION

The methanolic root extract of *Uncaria gambir* Roxb. exhibited concentration-dependent DPPH radical scavenging activity, with a mean IC_{50} value of 12.57 ± 0.04 µg/mL, indicating that gambir roots may serve as a promising source of natural antioxidants. Phytochemical screening showed the presence of phenolics, alkaloids, saponins, terpenoids, and flavonoids, whereas steroids were not detected. Although the antioxidant activity of the extract was lower than that of gallic acid as the positive control, the extract demonstrated notable antioxidant potential in the DPPH assay. Further studies are needed to identify the active constituents and to evaluate their antioxidant effects using additional *in vitro* and *in vivo* models.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research.

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