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ANTI-BREAST CANCER POTENTIAL AND WATER SOLUBILITY OF ETHYL ACETATE LEAF EXTRACT TEA FROM *Uncaria gambir* Roxb

POTENSI ANTI-KANKER PAYUDARA DAN KELARUTAN AIR DARI EKSTRAK DAUN TEH ETIL ASETAT DARI *Uncaria gambir* Roxb

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Abstract

The leaf extract of *Uncaria gambir* (UGR) was evaluated for its anticancer potential against 4T1 cells in vitro. Variations in soaking duration and mesh size affected extract yield, with 72-hour soaking and a mesh size of 60 producing the highest yield (32.6%). Polyphenolic compounds, particularly catechins, were identified as the main bioactive components, exhibiting dose-dependent cytotoxic effects, including induction of apoptosis, inhibition of cell proliferation, and modulation of oxidative stress. The IC_{50} value of the UGR leaf water extract in this study was 1006.2 µg/mL, higher than previous reports (87.7 µg/mL), likely due to differences in extraction methods, solvent type, and analytical protocols. Water solubility data are relevant to pharmaceutical characterization, given the solubility and bioavailability challenges of catechins. These findings suggest that optimizing extraction methods or using catechin-rich fractions can enhance the anticancer potential of UGR. The study supports the potential of UGR as a breast cancer chemopreventive agent, highlighting the importance of appropriate extraction techniques to maximize bioactive compound content.

Keywords: *Uncaria gambir, catechin, anticancer, leaf extraction, cytotoxicity*

Abstrak

Ekstrak daun Uncaria gambir (UGR) dievaluasi untuk potensi antikanker terhadap sel 4T1 secara in vitro. Variasi durasi perendaman dan ukuran mesh memengaruhi hasil ekstrak, dengan perendaman 72 jam dan mesh 60 menghasilkan hasil tertinggi (32,6%). Senyawa polifenolik, khususnya katekin, diidentifikasi sebagai komponen bioaktif utama yang menunjukkan efek sitotoksik dosis-bergantung, termasuk induksi apoptosis, penghambatan proliferasi sel, serta modulasi stres oksidatif. Nilai IC_{50} ekstrak air daun UGR dalam penelitian ini adalah 1006,2 µg/mL, lebih tinggi daripada laporan sebelumnya (87,7 µg/mL), yang kemungkinan disebabkan oleh perbedaan metode ekstraksi, jenis pelarut, dan protokol analisis. Data kelarutan dalam air relevan untuk karakterisasi farmasi, mengingat tantangan kelarutan dan bioavailabilitas katekin. Temuan ini menunjukkan bahwa pengoptimalan metode ekstraksi atau penggunaan fraksi kaya katekin dapat meningkatkan potensi antikanker UGR. Studi ini mendukung potensi UGR sebagai agen kemopreventif kanker payudara dan menyoroti pentingnya teknik ekstraksi yang tepat untuk memaksimalkan kandungan senyawa bioaktif.

Kata kunci: *Uncaria gambir*, katekin, antikanker, ekstraksi daun, sitotoksisitas

INTRODUCTION

The tradition of utilizing herbal plants as a fundamental part of traditional medicine persists in Indonesia, mirroring Ayurvedic practices in India and Traditional Chinese

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Medicine. This practice spans across social classes and geographic regions, from urban to rural areas, with approximately 40% of the population relying on it. Economically, Indonesia's medicinal plant industry is valued at 14.6 billion USD annually. Globally, the trade of medicinal plants is projected to soar to 5 trillion USD by 2050 (Cahyaningsih *et al*., 2021). This immense potential necessitates increased research into herbal plants, particularly to develop functional foods and beverages that support health and prevent chronic disease.

Breast cancer is a malignant tumor originating from breast cells that develop abnormally and invasively, with the potential to metastasize to other parts of the body. In Indonesia, breast cancer ranks as the most prevalent type of cancer and the leading cause of cancer-related mortality. Globocan 2020 data recorded 68,858 new breast cancer cases (16.6% of all new cancer cases), resulting in over 22,000 deaths (Ministry of Health, 2022). In West Kalimantan, the incidence of breast cancer is notably high, with approximately 441 recorded cases (Alkmail *et al*., 2023).

Although chemotherapy is often the primary treatment choice, the method carries adverse physical and psychological side effects, ranging from vital organ toxicity (heart, liver, and kidneys) and digestive disorders to a decline in patients' self-esteem and well-being (Sari *et al*., 2019). As an alternative to minimize these side effects, herbal tea from *Uncaria gambir* Roxb. (UGR) has emerged. In West Kalimantan, UGR is frequently cultivated for anti-cancer therapy due to its catechin content, which acts as an effective cytotoxic agent. Catechins work by triggering apoptosis in cancer cells and inhibiting angiogenesis and metastasis (Wardana *et al*., 2023).

In this study, UGR leaves were extracted with ethyl acetate. The selection of ethyl acetate was based on its semi-polar nature and low toxicity, which allows it to extract both polar and non-polar bioactive compounds (Melita *et al*., 2022). The efficacy of ethyl acetate extracts as potent anti-cancer agents has been supported by previous research on other plant species (Bajpai et al., 2024). The testing was conducted in two main stages: *in silico* analysis (utilizing PASS Online, Molecular Docking, and CADD) to predict activity, and *in vitro* testing using the MTT assay on 4T1 cancer cells. The 4T1 cell line was selected for its aggressive migratory and metastatic capabilities (Hidayati *et al*., 2022). Furthermore, the extract's water solubility was assessed to elucidate the chemical interactions among its compounds.

RESEARCH METHODS

The methodology applied in this study was adapted from Iskandar *et al*. (2022), with several modifications. The research was conducted in four main stages. First, in silico analysis was performed and evaluated using the PASS Server. Second, the leaves of *Uncaria gambir* (UGR) were extracted. Third, the extract's solubility in water was determined. Finally, in vitro testing was performed using the MTT Assay to assess the extract's biological activity.

The study utilized a range of equipment and materials to ensure accurate and reproducible results. The equipment included an oven, porcelain dishes, dropper pipettes, beakers, measuring cylinders, filter paper, funnels, Erlenmeyer flasks, filter cloths, a water bath, an evaporator, an analytical balance, a blender, a hammer mill, scissors, spatulas, sample bottles, and sieves with mesh sizes of 30, 40, and 50. The materials employed in the

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experiments comprised ethyl acetate, *Uncaria gambir* (UGR) leaves, gallon water, and distilled water. The methodology applied in this study was adapted from Iskandar *et al.* (2022) with several modifications. The research was carried out in four main stages. First, in silico analysis was performed and evaluated using the PASS Server. Second, the leaves of *Uncaria gambir* (UGR) were extracted. Third, the extract's solubility in water was determined. Finally, in vitro testing was conducted using the MTT Assay to assess the extract's biological activity.

In Silico Analysis

The in silico analysis was conducted to identify potential bioactive compounds in Uncaria gambir (UGR) and to predict their biological activities. The procedure began by accessing the KNApSAcK online database (http://www.knapsackfamily.com/KNApSAcK/) and searching for bioactive compounds using the keyword "*Uncaria gambir* plant." The retrieved data were organized in Microsoft Excel, after which the bioactive compounds were standardized by verifying their canonical SMILES using the PASS Online database (https://www.way2drug.com/). These verified canonical SMILES were then used to predict the compounds' biological activities, with particular focus on their potential anticancer properties (Malikhana *et al*., 2021).

UGR Leaf Extraction

Uncaria gambir (UGR) leaves were extracted with ethyl acetate to obtain the ethyl acetate extract for further analysis. Initially, 135 g of UGR leaves were weighed and ground using a blender and hammer mill. The resulting powder was sieved through mesh sizes of 20, 40, and 60, and 5 g of each sample was transferred into a jar containing 25 mL of ethyl acetate. The mixtures were macerated for 24, 48, and 72 hours, then pressed through a filter cloth and filtered through filter paper to remove impurities. The extracts were then concentrated in a water bath at 50˚C for 1 hour, weighed to determine the yield, and stored in labeled bottles as UGR ethyl acetate extract. The percentage yield was calculated using the formula: %Yield = (Mass of Dry Extract/5 g) × 100% (Iskandar *et al*., 2022).

Water Solubility Test

The water solubility of the *Uncaria gambir* (UGR) leaf extract was determined following the method of Marichelvam *et al*. (2019) with slight modifications. In this procedure, 15 g of the extract was dissolved in 50 mL and 100 mL of distilled water, and the mixture was continuously stirred for 6 hours. The solutions were then filtered through filter paper to separate the soluble fraction from the insoluble residue. The soluble extract was subsequently dried in an oven at 105 ℃ until a constant weight was reached, and the remaining dry extract was weighed to quantify the fraction dissolved in water. The total percentage of dissolved substances (WS%) was calculated using the formula WS% = (WO − WF) / WO × 100%, where WS represents water solubility, WO is the initial mass of the extract, and WF is the final mass of the dry extract.

In Vitro Assay

The in vitro testing was conducted following the method described by Elufioye *et al*. (2017). Cells were collected at a concentration of 8 × 10^3 cells per well and diluted using

culture medium. A 100 µL volume of the cell suspension was seeded into each well of a 96-well microplate and incubated for 24 hours in a 5% CO_2 incubator. Before treatment, the medium in the wells was removed, and the cells were washed once with 100 µL of phosphate-buffered saline (PBS) per well. PBS was then discarded, and test solutions at concentrations of 500, 250, 125, 62.5, and 31.25 ppm were added to each well to a final volume of 100 µL. The cells were incubated for another 24 hours, then washed with PBS, and 100 µL of MTT reagent was added to each well, followed by incubation for 3–4 hours at 37 ℃. Subsequently, 100 µL of stopper solution (10% SDS in 0.01 N HCl) was added to each well, and the plate was incubated overnight at room temperature (25 ℃) in the dark. The absorbance was measured at 595 nm using an ELISA reader to determine the viability of 4T1 or Vero cells. The absorbance data were then converted to percentage cell viability, which was used to calculate the IC_{50} value.

Cell Viability and Selectivity Index (SI) Determination

Cell viability was calculated using the following formula: % Cell Viability = (A_sample / A_control) × 100%. The selectivity index (SI) was determined by dividing the IC_{50} value of Vero cells by the IC_{50} value of 4T1 cells, as expressed by the equation: SI = IC_{50} Vero cells / IC_{50} 4T1 cells (Indrayanto *et al*., 2021).

Data Analysis

Data analysis was conducted using descriptive statistics to characterize the data. Graphs or diagrams were created to visualize the data, which helped in identifying patterns or trends. Descriptive statistical calculations included measures such as the mean, median, and mode, as well as measures of dispersion, including range, variance, and standard deviation.

Research Design

The research was conducted using a Completely Randomized Design (CRD) with a 3 × 3 factorial arrangement. The first factor was the mesh size used for sieving the UGR leaves, consisting of 20 (U1), 40 (U2), and 60 (U3) mesh. The second factor was the soaking duration of the leaves, including 24 hours (L1), 48 hours (L2), and 72 hours (L3). Each combination of factors was replicated three times, resulting in a total of 27 experimental units. Table 2 summarises the experimental design, where each sample code (e.g., L1U1) represents a specific combination of soaking duration and mesh size.

RESULTS AND DISCUSSION

1. **Results**

Calculation of UGR Leaf Extraction Results

The extraction of *Uncaria gambir* (UGR) leaves was carried out under varying soaking durations and mesh sizes. Each combination of factors—soaking duration (24, 48, and 72 hours) and mesh size (20, 40, and 60)—was replicated three times, and the extract mass for each replicate (U1, U2, and U3) was recorded. The average extract mass for each sample code (e.g., L1U1, L2U2) was then calculated, and the extraction yield (%) was determined. For instance, the 24-hour soaking with mesh 20 (L1U1) resulted in an average

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extract mass of 0.86 g and a yield of 17.2%, while the 72-hour soaking with mesh 60 (L3U3) yielded 32.6% with an average mass of 1.63 g. This approach enabled a systematic comparison of extraction efficiency across treatment conditions and provided a clear overview of how soaking duration and mesh size influence the yield of UGR leaf extracts.

Calculation of Water Solubility

The water solubility of the UGR leaf extract was determined by dissolving 15 g of extract in 50 mL and 100 mL of distilled water. The weights of the empty dish, the dish with the dissolved sample, and the initial extract were recorded to calculate the amount of extract that dissolved in water. For the ethyl acetate extract, the dissolved fraction in 50 mL of water was 0.81352 g, while in 100 mL it was 1.6270 g. These measurements enabled the determination of the extract's solubility in water under the tested conditions.

Anticancer Assay

The anticancer activity of the UGR leaf extract was evaluated by measuring cell viability at various concentrations (62.5, 125, 250, 500, and 1000 µg/mL). Absorbance readings for three replicates (A1, A2, A3) were recorded, and the mean absorbance values were used to calculate the corresponding cell viability percentages (V1, V2, V3). For example, at the highest concentration of 1000 µg/mL, the mean absorbance was 0.429, corresponding to a cell viability of 40.77% with a standard deviation of 2.39 and a standard error of 1.38. At lower concentrations, cell viability increased, reaching 96.92% at 250 µg/mL. Control readings were also measured, with the cell control showing a mean absorbance of 0.977 and the media control 0.051. These results provided a quantitative assessment of the extract's cytotoxic effects and enabled the calculation of IC_{50} values for anticancer evaluation.

IC_{50} Value

The graph illustrates the relationship between concentration (µg/L) and cell viability (%) as a function of concentration. The linear regression equation is y = -0.0526x + 102.93. Where Y represents cell viability, and X represents concentration. Substituting Y = 50 to determine X:

$$50 = -0.0526x + 102.93 \quad \Rightarrow \quad X = \frac{102.93 - 50}{0.0526} \approx 1006.2\,\mu g/mL$$

Thus, the IC_{50} value is 1006.2 µg/mL.

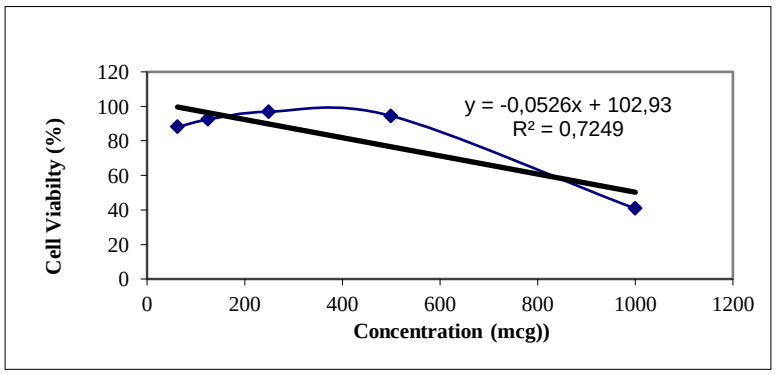

Figure 1. Assessment of 4T1 Cell Viability after Treatment

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2. Discussion

Analysis of UGR Leaf Extraction Results

The extraction of *Uncaria gambir* (UGR) leaves was carried out under varying soaking durations and mesh sizes, resulting in significant variations in yield. The soaking duration and particle size affect the surface area of particles in contact with the solvent, influencing the amount of bioactive compounds extracted (Palos-Hernández *et al*., 2024). Longer soaking times and finer mesh sizes (higher mesh number) typically produce higher extract yields due to increased contact between the plant matrix and solvent, which facilitates the release of phenolic compounds such as catechins—a class of polyphenols abundant in *Uncaria gambir* (Palos-Hernández *et al*., 2024). In the present results, the combination of 72 hours of soaking with mesh 60 provided the highest extract mass and yield (1.63 g, 32.6%). Controlling parameters such as time, temperature, particle size, and solvent ratio is standard practice in optimizing extraction processes to maximize the recovery of bioactive compounds (Sun *et al*., 2025). Recent studies on extraction optimization emphasize that systematic variation and careful control of process conditions are critical for achieving maximum yield and bioactivity in plant extracts (Sun *et al*., 2025).

Discussion of Water Solubility

Determining water solubility is an important step in extract characterization because it affects pharmaceutical formulation and bioavailability. Polyphenolic compounds such as catechins are generally polar and water-soluble, but at high concentrations they may aggregate, reducing apparent solubility. Recent literature indicates that a major challenge in utilizing polyphenols for therapeutic applications is their poor aqueous solubility and limited bioavailability, which can significantly restrict their effectiveness in vivo (Sahraeian, 2024; Lu *et al*., 2025). Various nanotechnology-based delivery systems, such as nanoemulsions and other nanocarrier approaches, have been shown to improve the stability, solubility, and bioavailability of polyphenols by protecting them from degradation and enhancing their digestive and absorptive profiles (Li *et al*., 2023; Wang *et al*., 2025). These nano-delivery strategies aim to overcome limitations in dissolution and absorption, thereby increasing the potential for polyphenols to exert their biological effects when administered orally or topically (Li *et al*., 2023; Wang *et al*., 2025).

Analysis of Anticancer Assay

The in vitro anticancer assay against 4T1 cells showed that UGR leaf extract exhibited dose-dependent cytotoxic effects, with cell viability decreasing at higher concentrations. Polyphenols such as catechins are the main bioactive compounds responsible for anticancer activity via multiple mechanisms, including induction of apoptosis (programmed cell death), inhibition of cell proliferation, and modulation of oxidative stress in cancer cells. Recent studies (Iskandar *et al*., 2024) report that water extracts of UGR leaves exhibit significant cytotoxic potential against 4T1 cells, with an IC_{50} of approximately 87.7 µg/mL, which is much lower than the IC_{50} value obtained here (1006.2 µg/mL). This difference may result from extraction methods, solvent types, or analytical protocols. Other studies on MCF-7

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human breast cancer cells showed that ethyl acetate fractions rich in catechins exhibited stronger cytotoxic activity (IC_{50} ~87.5 µg/mL), supporting the role of catechin concentration in anticancer effectiveness.

Discussion of IC_{50} Value

The IC_{50} (Inhibitory Concentration 50%) is a key parameter for evaluating the anticancer potential of an extract. The IC_{50} of 1006.2 µg/mL indicates relatively lower cytotoxic activity compared to other studies, such as IC_{50} ~87.7 µg/mL for 4T1 cells with water extract of UGR leaves (Iskandar et al., 2024) and IC_{50} ~87.5 µg/mL for MCF‑7 cells with ethyl acetate fractions. These differences may arise from the extraction method and solvent type, catechin content, and variations in cell lines or assay protocols. Nonetheless, despite the higher IC_{50} (lower activity), the extract still showed a dose-dependent decrease in cell viability, consistent with the dose-response cytotoxicity pattern reported in other studies.

CONCLUSION

The relationship between extraction parameters and yield is consistent with the literature, which shows that soaking time and mesh size affect extract yield by increasing solvent-powder contact. Water solubility results are relevant to pharmaceutical characterization and are consistent with the solubility challenges of bioactive flavonoids in formulations. Cytotoxic effects on 4T1 cells align with catechin activity reported in other studies. However, the relatively high IC_{50} suggests a need to optimize extraction methods or use catechin-rich fractions to enhance anticancer potential. Studies from 2024–2025 indicate that Uncaria gambir has potential against breast cancer, particularly when bioactive compounds are maximized through appropriate solvent extraction or fractionation.

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