Jurnal Veteriner dan Biomedis
Vol 3, No 2 (2025): 130-133. 10.29244/jvetbiomed.3.1.130-133
https://journal.ipb.ac.id/index.php/jvetbiomed
e-ISSN 2987-2251, p-ISSN 2985-4954

PENELITIAN/RESEARCH OPEN ACCESS

Cytotoxicity of Gambir Leaf Infusion (*Uncaria gambir* Roxb.) on T47D Cells: In Vitro Assay

Amaq Fadholly*
Division of Pharmacology and Toxicology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia

Received: 12/04/2025, Accepted: 21/08/2025, Published Online: 30/09/2025
*Corresponding author: amaqfadholly@apps.ipb.ac.id

ABSTRACT

Cancer is a cellular pathology marked by the disruption of regulatory mechanisms governing the cell cycle and homeostasis in multicellular organisms. This study seeks to evaluate the cytotoxic effects and IC_{50} value of *Uncina gambir* leaf infusion on the T47D cell line, utilized as a model for mammary cancer. The cytotoxic activity of *Uncina gambir* leaf extract was assessed by delivering five concentrations of the test substance: 10 µg/ml, 20 µg/ml, 40 µg/ml, and 80 µg/ml to T47D cell lines, followed by a 24-hour incubation period. Cell counting was conducted following the administration of MTT and SDS stop solution. The inhibition percentages for each test sample concentration were 25.11%, 33.76%, 43.79%, and 48.09%, respectively. The extract of *Uncina gambir* leaf infusion exhibits an IC_{50} value of 502.12 µg/mL. The IC_{50} value of *Uncina gambir* leaf infusion against T47D cell lines acts as non toxic for the cytotoxicity group. Consequently, it may be inferred that *Uncina gambir* leaf extract exhibits no cytotoxicity towards T47D cell lines.

Keywords: Cancer, Cytotoxicity, Gambir, T47D

1. Introduction

Cancer ranks among the primary causes of mortality globally. Breast cancer constitutes a significant health issue, predominantly affecting women[1][2]. The WHO reported that in 2020, there were over two million breast cancer diagnoses and more than 600,000 breast cancer-related fatalities[3]. Breast cancer is a hereditary condition resulting from the accumulation of genetic mutations in breast tissue. Various breast cancer cell types are utilized in in vitro cancer research, including T47D cells, which are a human ductal epithelial carcinoma cell line. Cancer issues can typically be addressed through the excision of malignant tissue or by eradicating cancer cells while mitigating adverse effects on healthy cells. This must be matched with the treatment of chemotherapy or radiation to mitigate the risk of metastasized cells and to suppress the proliferation of any residual cancer cells. Patients undergoing breast cancer treatment with the combination of the anticancer agents 5-flouracil, doxorubicin, and cyclophosphamide exhibited hair loss, nail discolouration, alterations in taste, reduced appetite, nausea, and neurological disturbances[4].

Continued efforts are necessary to discover safe and effective pharmaceuticals, one approach being natural exploration. Researchers have excavated and investigated its natural resources to identify prospects for generating novel pharmaceuticals. Nevertheless, to date, the chemical composition, effectiveness, and adverse effects of therapeutic plants have not been well documented. Plants serve as therapeutic agents owing to their diverse biological actions, attributed to the existence of secondary metabolites, including alkaloids, terpenoids, steroids, saponins, flavonoids, and polyphenols[5][6]. Gambir (*Uncaria gambir* Roxb) is a plant known for its therapeutic benefits. Gambier sap has been extensively utilized for the treatment of several diseases, including gout, fever, diabetes, diarrhea, headaches, colds, and coughing[7][8]. Gambier sap has historically been utilized for illness treatment; nevertheless, there is a paucity of in vitro studies on cell lines demonstrating its efficacy in inhibiting cancer cell proliferation. Consequently, additional research is required to investigate the action of gambir sap in cell lines. This research seeks to ascertain the IC_{50} value of gambir leaf infusion utilizing the T47D cell line.

2. Materials and methods

2.1. Ethical Statement

All treatments and procedures in this study have received approval from the Medical and Health Research Ethics Commission, Faculty of Medicine, Public Health and Nursing, Gadjah Mada University, Indonesia (No: KE/FK/0106/EC/2018).

2.2. *Uncina gambir* Infusion Preparation

Leaves of *Uncina gambir* were procured from Malang Regency, East Java, Indonesia. The intact green soursop leaves were subsequently wet sorted to eliminate foreign materials and impurities, then thoroughly washed, cut into 10-gram pieces, placed into an infusion pan, combined with 100 ml of distilled water, and heated for 15 minutes at an initial temperature of 90°C, with occasional stirring. The filtered infusion is supplemented with distilled water to reach a total volume of 100 ml.

2.3. Culture of T47D cell line

T47D cell lines were acquired from the Parasitology Laboratory at the Faculty of Medicine, Gadjah Mada University, Indonesia. Cells were grown in DMEM media augmented with 10% (v/v) FBS, 3% streptomycin-penicillin, and 1% fungizone, and incubated in a 5% CO2 incubator at 37°C. Cells were grown in 25 cm^2 flasks containing 7 ml of media and collected with 0.25% trypsin-EDTA at attaining 80% confluence.

2.4. Calculation of IC_{50} Value

A test solution was created by dissolving 10 mg of *Uncaria gambir* leaf infusion in 100 µl of DMSO. A series of concentrations 10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL were produced from the stock solution in DMEM culture media for cytotoxicity assessment, conducted in triplicate with cisplatin as a positive control. The IC_{50} value of *Uncaria gambir* leaf infusion against T47D cell lines was ascertained by MTT assay findings. Cells were cultivated in 96-well plates at a density of 1x10^4 cells per well in a volume of 100 µL and incubated at 37°C with 5% CO2 overnight. Cells were treated with varying doses for 24 hours, after which the medium was changed with 100 µL DMEM and 10 µL MTT (5 mg MTT/mL) each well and incubated for 4 hours. The control cell treatment involved merely medium without any

intervention. Formazan crystals generated in living cells were solubilized with 100 µL of 0.1 N SDS hydrochloric acid and quantified using an enzyme-linked immunosorbent assay (ELISA) reader (Bio Rad, USA) at an absorbance of 595 nm.

2.5. Data analysis

Absorbance and IC_{50} value discrepancies between the treatment and control groups were determined by linear regression analysis utilizing Microsoft Excel 2020 (Microsoft Inc., USA).

3. Results

The cytotoxicity test findings of *Uncaria gambir* leaf infusion indicated that an increase in the concentration of the infusion corresponded with a decrease in the viability of T47D cell lines. The maximum suppression of T47D cell line proliferation induced by *Uncaria gambir* leaf infusion was observed at a dose of 80 µg/mL, yielding an inhibition value of 48.09%, while the minimum value was recorded at a concentration of 10 µg/mL, resulting in an inhibition value of 25.11%. The inhibition values at doses of 20 and 40 µg/mL were 33.76% and 43.79%, respectively, resulting in an IC_{50} value of 502.12 µg/mL for *Uncaria gambir* leaf infusion against T47D cell lines. According to the inhibitory value, *Uncaria gambir* leaf infusion has not attained a growth inhibition of the T47D cell line of 50% or above. These results differ with the observations of T47D cell line proliferation when treated with doxorubicin as a positive control. The inhibition rates at identical concentrations, ranging from low to high, were 45.32 %, 55.98%, 60.81%, and 72.73%, with an IC_{50} value of 86.14 µg/mL. The data on the average inhibition rates for T47D cell lines after treated *Uncaria gambir* leaf infusion presented in **Table 1**.

4. Discussion

This study demonstrates that *Uncaria gambir* leaf infusion inhibits the proliferation of T47D cell lines, with the degree of inhibition increasing in accordance with the concentration administered, however, it has not achieved the threshold of 50% inhibition. According to the classification of toxic compound categories in natural product toxicity research, there are four classifications: the highly toxic category for IC_{50} values ≤ 20 µg/mL, the moderately toxic category for IC_{50} values between 21–200 µg/mL, the weakly toxic category for IC_{50} values between 201–500 µg/mL, and the non-toxic category for IC_{50} values ≥ 500 µg/mL[9]. According to the IC_{50} value of 502.12 µg/mL, *Uncaria gambir* leaf infusion is classified as non toxic or exhibits limited efficacy in inhibiting and eradicating T47D cell lines. Multiple prior research have demonstrated non-toxic outcomes, including the application of *Annona squamosa* leaf infusion to HeLa cell lines, curly chili leaf extract to HeLa cell lines and Allium cepa extract to WiDr cell lines[10][11][12].

The findings presented in **Table 1** indicate that the growth-inhibitory effects on T47D cell lines are linked to the metabolite chemicals found in *Uncaria gambir* leaves. The alkaloid content can influence signaling pathways related to proliferation, the cell cycle, and metastasis. These chemicals function by producing DNA damage, triggering apoptosis, and serving as anti-proliferative agents. Alkaloids can enhance apoptosis by causing DNA damage[13]. Alkaloid substances suppress proliferation by obstructing oxidative mechanisms that may initiate cancer. This process is facilitated by a reduction in the enzymes lipoxygenase, cyclooxygenase, and xanthine oxidase, which are essential for the pre-oxidation process, influencing the cell cycle[14]. Steroid activity exhibits harmful effects on cells, including HeLa cells. Moreover, steroids exhibit

Table 1. IC_{50} values of *Uncaria gambir* leaf infusion and cisplatin on T47D cell lines.

Sample	Concentration (µg/mL)	Cell Growth Inhibition (%)	IC_{50} (µg/mL)
Uncaria gambir leaf infusion	10	25.11	502.12
	20	33.76	
	40	43.79	
	80	48.09	
Doxorubicin	10	45.32	86.14
	20	55.98	
	40	60.81	
	80	72.73	

anti-tumor properties against several cancer cell types by influencing the G1/S phase of the cell cycle. The G1 phase significantly impacts the cell cycle. If cells in the G1 phase opt to proceed with the cell cycle, they will transition into the subsequent stage, the S phase. Consequently, steroid chemicals impede the growth of cancer cells by obstructing cell development in the G1 phase[15]. The flavonoids included in *Uncaria gambir* leaf are classified as polyphenolic chemicals, which are secondary metabolites exhibiting anticancer properties[16]. Flavonoids encompass quercetin, derived from the flavonol subclass. Quercetin, genistein, and flavopiridol may serve as components in cancer therapeutics. Flavonoids enhance enzyme activity, hence facilitating apoptosis, disrupting the cell life cycle, modulating immunological function, and suppressing inflammation, angiogenesis in cancer cells, and proliferation[17][18]. The findings from the efficacy assessment of *Uncaria gambir* leaf infusion in suppressing T47D cell line indicated that the bioactive constituents in *Uncaria gambir* leaf might diminish T47D cell growth, however, less effectively than doxorubicin.

5. Conclusion

The infusion of *Uncaria gambir* leaves can suppress the proliferation of HeLa cell lines by 48.09% at a maximum concentration of 80 µg/mL, with an IC_{50} value of 502.12 µg/mL. According to the inhibitory value, *Uncaria gambir* leaf infusion has not demonstrated an inhibition of T47D cell line growth over 50%, indicating that its administration in this study has not been substantiated as effective in inhibiting T47D cell line proliferation. The infusion of *Uncaria gambir* leaf requires further development in various formulations and administration to different cell types to ascertain the unique potential of this preparation.

References

[1] **Akram M, Iqbal M, Daniyal M, & Khan AU.** 2017. Awareness and current knowledge of breast cancer. Biol. Res. 50(1): 33.

[2] **Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, et al.** 2017. Risk factors and preventions of breast cancer. Int. J. Biol. Sci. 13(11): 1387–1397.

[3] **DeSantis CE, Bray F, Ferlay J, Lortet-Tieulent J, Anderson BO, & Jemal A.** 2015. International variation in female breast cancer incidence and mortality rates. Cancer Epidemiol. Biomarkers Prev. 24(10): 1495–1506.

[4] **Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, et al.** 2022. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis. 10(4): 1367–1401.

[5] **Saragih DE, & Arsita EV.** 2019. Kandungan fitokimia Zanthoxylum acanthopodium dan potensinya sebagai tanaman obat di wilayah Toba Samosir dan Tapanuli Utara, Sumatera Utara. Pros Sem Nas Masy Biodiv Indon. 5(1): 71–76.

[6] **Nasim N, Sandeep IS, & Mohanty S.** 2022. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus (Calcutta). 65(3): 399–411.

[7] **Oswari L, Hidayat R, Fatmawati F, Hayati L, & Alisa BS.** 2019. Gambir extract (*uncaria gambir*) decreases inflammatory response and increases gastric mucosal integrity in wistar rats - model gastritis. Open Access Maced. J. Med. Sci. 7(19): 3149–3152.

[8] **Auliana FR, Ifora I, & Fauziah F.** 2022. Phytochemical and anti-inflammatory of Uncaria gambir: a review. Asian Journal of Pharmaceutical Research and Development. 10(1): 79–83.

[9] **Makia R, Al-Sammarrae K, Al-Halbosiy M, & Al-Mashhadani M.** 2022. In vitro cytotoxic activity of total flavonoid from *equisetum arvense* extract. Rep. Biochem. Mol. Biol. 11(3): 487-492.

[10] **Fadholly A.** 2024. Sitotoksisitas ekstrak cabe merah keriting (*Capsicum annum*) terhadap hela cell lines. Jurnal Veteriner dan Biomedis. 2(1): 56–60.

[11] **Fadholly A, Ansori ANM, Jayanti S, Proboningrat A, Kusala MKJ, Putri N, Rantam FA, & Sudjarwo SA.** 2019. Cytotoxic of *Allium cepa* L. on human colon cancer (WiDr) cells: in vitro study. Research Journal of Pharmacy and Technology. 12(7): 3483–3486.

[12] **Fadholly A.** 2024. Sitotoksisitas infusa daun srikaya (Annona squamosa) terhadap HeLa cell lines. Jurnal Veteriner dan Biomedis. 2(2): 106–111.

[13] **Habli Z, Toumieh G, Fatfat M, Rahal ON, & Gali-Muhtasib H.** 2017. Emerging cytotoxic alkaloids in the battle against cancer: overview of molecular mechanisms. Molecules. 22(2): 250.

[14] **Hashmi MA, Khan A, Farooq U, & Khan S.** 2018. Alkaloids as cyclooxygenase inhibitors in anticancer drug discovery. Curr. Protein Pept. Sci. 19(3): 292–301.

[15] **Bertoli C, Skotheim JM, & de Bruin RA.** 2013. Control of cell cycle transcription during G1 and S phases. Nat. Rev. Mol. Cell. Biol. 14(8): 518–28.

[16] **Munggari IP, Kurnia D, Deawati Y, & Julaeha E.** 2022. Current research of phytochemical, medicinal and non-medicinal uses of *uncaria gambir* roxb.: a review. Molecules. 27(19): 6551.

[17] **Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, et al.** 2020. Important flavonoids and their role as a therapeutic agent. Molecules. 25(22): 5243.

[18] **Alharbi HOA, Alshebremi M, Babiker AY, & Rahmani AH.** 2025. The role of quercetin, a flavonoid in the management of pathogenesis through regulation of oxidative stress, inflammation, and biological activities. Biomolecules. 15(1):151.

