Anticancer activity of *uncaria gambir roxb* on T47D breast cancer cells

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**Abstract.** Breast cancer was the second most common cancer that causes death in women worldwide. One ingredients of herbal plants as an alternative therapy in the community, was gambir leaves. This research was conducted to examine the anticancer activity of *Uncaria gambir Roxb* on T47D breast cancer cells in vitro. Six levels of concentration *U. gambir* (31.25 mg/ml up to 1000 mg/ml) was included on the 96 microplate well which had been filled with breast cancer cells, the positive control was doxorubicin (with concentration of 0.03-1 mg/ml). The anticancer effects of *U. gambir* was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-diphenyl tetrazolium bromide 2.5). The results showed the existence of anticancer activity of *Uncaria gambir Roxb* at concentrations of 250, 500 and 1000 µg/ml. The largest anticancer activity found at a concentration of 500 µg/ml with the percentage of apoptotic cells of breast cancer by 20%, but we also found a decrease in the value of the apoptotic at concentration of 1000 µg/ml. The IC50 of *U. gambir* was 1.086 µg/ml whereas the IC50 doxorubicin was 0.108 µg/ml. This study concluded that the inhibition of breast cancer cell growth by gambir leaves extract was still quite weak compared to doxorubicin.

1. **Introduction**

Breast cancer was the second most common cancer that causes death in women worldwide. The incidence of breast cancer increased every year, especially in developing countries due to the increased of life expectancy, lifestyle, urbanization [1]. Previous studies showed the pathogenesis of breast cancer linked with the production of reactive oxygen species (ROS) such as superoxide anion, singlet oxygen, hydrogen peroxide (H2O2) [2] [3]. Several studies have shown the plant products or plant extract have antioxidant effect which scavenge free radical [4].

*Uncaria gambir Roxb* was belong to Rubicaceae family, this plant was popular as complementary medicine in Asia especially Indonesia and Malaysia due to anti-inflammatory and antioxidant effect. In Indonesia, this plant can mostly be found in West Sumatera, North Sumatera, Riau and South Sumatera [5]. Traditionally, *U. gambir* was used as treatment for diarrhoea, wounds and ulcers, fevers,
Headaches, gastrointestinal illnesses and bacterial/fungal infections, sore throat [6]. The major flavonoid in *U. gambir* is catechin followed by epicatechin and alkaloid [7]. Previous studies already investigate the effect of *U. gambir* as antihyperlipidemia, antiatherosclerosis, but limited studies showed anticancer effect of *U. gambir* due to antioxidant properties [8]. However, this research aim to investigate anticancer activity of *U. gambir* in vitro.

2. Method
This research was in vitro study. This study was conducted on May 2018 in Parasitology Laboratorium, Faculty of Medicine, Gajah Mada University. The study was conducted after obtaining approval from the ethics committee of the Faculty of Medicine, University of Sumatera Utara (USU) with the letter of ethical approval No. 386/FK USU.

2.1. Reagents
*U. gambir* was bought from the Toyo Brothers company (Jakarta, Indonesia) mixed with dimethyl sulfoxide (DMSO) at a concentration of 10 mM, after going through a dilution process, extracted with 6 different concentrations of 15,625; 31.25; 62.5; 125; 250; 500; up to 1000 µg / ml which is put into a tube that contains medium culture cells. 10% FBS (Fetal Bovine Serum), 1-2% Penstrep (Penicillin-Streptomycin), 0.5%: Amphotericin B, PBS (Phosphate Bovine Serum), Tripsin-EDTA 0.25% that prepared from the Parasitology Laboratory, Faculty of Medicine UGM, Indonesia.

2.2. Cell Line And Culture
The human breast cancer cell line used was T47D. T47D cells were cultivated in culture flasks in RPMI media which supplemented with 10% FBS (Fetal Bovine Serum), 1-2% Penstrep (Penicillin-streptomycin), 0.5%: Amphotericin B, and then was stored in an incubator at -80°C with liquid nitrogen.

2.3. MTT ASSAYS
Cell viability was assessed using MTT examination. T47D breast cancer cells in the RPMI medium and T47D breast cancer cells that contained *U. gambir* in different concentrations in 96-well plates that had been incubated overnight, then the medium discarded, washed with Phosphate Buffer Serum and added with 100 µL MTT (Concentration 0.5 mg / ml), incubated 6 hours in a CO2 incubator, then added 100µL SDS 10%, stored in the incubator for 1 night. Examination of absorbance was assessed using a microplate reader with λ is 595 ms. Calculation of percentage of living cells (cell viability) is calculated using the following equation:

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\text{%Cell viability} = \left( \frac{\text{Sample Abs.} - \text{Media Control Abs.}}{\text{Sample control cell.} - \text{Media Control Abs.}} \right) \times 100\% 
\]

3. Results and Discussions
In the administration of *U. gambir* at a concentration of 1000 µg / ml, it was found that the percentage of living breast cancer cells was 93.6% while in the administration of *U. gambir* at a concentration of 500 µg / ml, a percentage of living cancer cells decreased to 80%. At a concentration of 500 µg / ml *U. gambir*, breast cancer cell death occurred by 20%. When the concentration of *U. gambir* was reduced to 250 µg / ml, there was a percent increase in living cells to 92%. At the time of administration of *U. gambir* with a concentration of 125; 62.5; 31.25 µg / ml, there was no decrease in the percentage of real breast cancer cells. Comparison of the percentage of living cells in various concentrations of *U. gambir* is shown in Figure 1.
In the administration of doxorubicin with a concentration of 1 µg/ml, we found a percentage of living cells by 39%, when the concentration was reduced to 0.5 µg/ml, there was a significant decrease in the percentage of living cells up to 9.47%. In the doxorubicin concentration of 0.25 µg/ml, we found the percentage of living cells was 10%. At a concentration of 0.125 µg/ml, there was an increase in the percentage of living cells to 39%, as well as when the concentration of doxorubicin was reduced to 0.0625; 0.03125 µg/ml, we found an increase in the percentage of living cells, about 74% and 92%. The 50% values of doxorubicin inhibitor concentration (IC50) in T47D cells is 0.108 µg/ml. Comparison of the percentage of living cells in various concentrations of doxorubicin is shown in Figure 2.

In the present research, we found the differentiation of cell viability between *U. gambir* and doxorubicin treatment. In figure 3, the T47D breast cancer cells still alive and only 6.4% died after the treatment of *U. gambir* with concentration 1000 µg/ml.
After the administration of 500 µg/ml Uncaria gambir Roxb, the T47D breast cancer cells died quite significant. It can be seen in figure 4.

After the administration of doxorubicin with a concentration of 1 µg / ml, we can see many T47D breast cancer cells died. It can be seen in figure 5.

In this research, we can see that flavonoid especially catechin in Uncaria gambir Roxb have antioxidant activity [9]. The mechanism of action of antioxidant effect by scavenging the free radical. Superoxide anions damage cells by forming OH,H202, singlet oxygen and peroxynitrit during aging and pathological events include cancer. Hydroyxyl radical such as OH,singlet oxygen attacks DNA, protein,fatty acids in cell membrane. Nitric oxide involve in inflammation, cancer and pathological condition [10]. In the research of Amir et al showed Uncaria gambir can be a natural antioxidant [11].

The results showed that Uncaria gambir in decreased concentration (below 500 µg/ml) did not have an effect to apoptotic the breast cancer cells. This could be happened due to pathophysiology of breast...
cancer was complex. Prenatal conditions, diet, estrogen exposure, a positive family history, can be the risk factors. The risk will increase if the woman get prolonged exposure with endogenous estrogen. The differentiation of estrogen and progesterone receptor subtypes based ethnic also affect the possibility of breast cancer [12]. Doxorubicin (DOX) is an anthracycline antibiotic commonly used in breast cancer chemotherapy, this drug work not only by scavenging free radical but also intercalating DNA. DNA will damage after doxorubicin intercalate the DNA and inhibit topoisomerase II [13].

4. Conclusions
In this research shows that the inhibition of breast cancer cell growth by *U. gambir* was weak compared to doxorubicin.

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