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Original Research Article

Cytotoxic Activity Screening of Various *Uncaria Spp* Plants on T47d Breast Cancer Cells

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ABSTRACT

Breast cancer is a disease characterised by uncontrolled cell growth in breast cells. One of the plants known to have anticancer activity is a plant from *Uncaria* genus. Some of those found in Province "Riau", Indonesia include *Uncaria barbata* Merr (UB), *Uncaria cordata* (Lour) Merr (UC), *Uncaria guianensis* (Aubl.) J.F.Gmel (UG) and *Uncaria nervosa* Elmer (UN). This study aimes to determine the cytotoxic effect of ethanolic extract from the leaves and stem bark of *Uncaria Spp* on T47D breast cancer cells. These were macerated using 70% ethanol and evaporated to obtain a viscous extract. The cytotoxic effect was determined using an MTT assay, and the IC_{50} value was identified after 48 hours of incubation. The results show that the IC_{50} values of the ethanolic extracts of the leaves of *Uncaria barbata* Merr , *Uncaria cordata* (Lour) Merr , *Uncaria guianensis* (Aubl.) J.F.Gmel, and *Uncaria nervosa* Elmer were 227.30, 653.13, 334.65, and 64.42 µg/mL, respectively. The corresponding values of the ethanolic stem bark extracts of *Uncaria cordata* (Lour) Merr and *Uncaria nervosa* Elmer were 124.45 and 526.02 µg/mL, respectively. The ethanolic extract of *Uncaria nervosa* leaves had a strong cytotoxic effect on T47D breast cancer cells, which was 64.42 µg/mL.

Keywords: *Uncaria Spp*, breast cancer, T47D cells, MTT assay method .

Introduction

Women are commonly affected by breast cancer, which often results in death. The disease is precipitated by disruption to the genes that control growth and differentiation, which allows these cells to proliferate and develop quickly and uncontrollably. According to Globocan data from 2020, Indonesia has 16.6% of the world's breast cancer cases.[1] Breast cancer treatment is widely given, but drug resistance is one of the main associated problems.[2]

Previous research on the plant from Sumatra has been conducted. The results show that some of these have the potential to be developed into novel medications.[3-9] One of the Sumatran plants with the potential to be used as an anticancer medication is *Uncaria*. *Uncaria guianensis* (Aubl.) J.F.Gmel, *Uncaria tomentosa*, *Uncaria gambir* and *Uncaria nervosa* are some of the *Uncaria* species that have been reported to have cytotoxic effects. *Uncaria guianensis* (Aubl.) contains polyphenols, kaempferitrin, uncarine C, and E and because of its cytotoxic properties, can damage DNA RS321 and RS322.[10,11] *Uncaria tomentosa* has an antiproliferative effect against adenocarcinoma of the colon (SW620), breast cancer (MCF7) and gastric cells (AGS),[12] and can increase ROS production in HepG2 cells, resulting in a decrease in GSH levels, meaning apoptosis occurs.[13] *Uncaria gambir* (Hunter) Roxb has cytotoxic activity on T47D7 breast cancer cells.[14] *Uncaria nervosa* Elmer contains alkaloids, flavonoids, terpenoids, and phenolics, and the results of the BSLT test are LC_{50} 1.76 ppm.[15,16]

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Additionally, plants from the *Uncaria* genus have been utilised to cure cancer in Indonesia, particularly in Province "Riau". A Species that has been used traditionally by local people is *Uncaria cordata*, with the leaves and bark of the plant used for cancer therapy. However, the effective use of the plant needs to be scientifically proven in order for it to be developed into a new alternative medicine based on local knowledge. In this research, we also considered other species growing in different locations and geographical conditions. The selection of these species took into account easy access to the collection locations, their availability for development, and if they had the potential to be cytotoxic. Some of the species collected were *Uncaria barbata* Merr, *Uncaria cordata* (Lour) Merr., *Uncaria guianensis* (Aubl.) J.F. Gmel and *Uncaria nervosa* Elmer. Four types of leaves and bark were employed in this research. Each sample was macerated using 70% ethanol solvent, and cytotoxic testing was performed using the MTT method.

Materials and Methods

Plant material

Leaves and bark of the plants *Uncaria barbata*, *Uncaria cordata* (Lour.) Merr, *Uncaria guianensis*, and *Uncaria nervosa* were obtained in forest areas in Pekanbaru, Kampar Regency and Rokan Hulu Regency, Province "Riau", Indonesia. Sample identification was conducted at the Herbarium ANDA Andalas University Indonesia in November 2022, with the samples coded NR 001, NR 002, NR 003 and NR 004. The samples were of eight types; ethanolic extract of the leaves of *Uncaria barbata* (DUB), *Uncaria cordata* (Lour.) Merr (LUC), *Uncaria guianensis* (LUG) and *Uncaria nervosa* (LUN); and the bark of *Uncaria barbata* (SBUB), *Uncaria cordata* (Lour) Merr (SBUC), *Uncaria guianenis* (SBUG) and *Uncaria nervosa* (SBUN). Human breast cancer T47D cell lines were obtained from the Tissue Culture Laboratory of Medicine Gajah Mada University, Yogyakarta, Indonesia.

Extraction

The samples were cleaned by washing them under running water, then dried. Subsequently, they were re- weighed, and the simplicia mashed

with a blender to an acceptable degree of fineness.[17] Dry powder (1:10) was placed into a dark-coloured bottle and soaked in 70% ethanol for 6 hours with occasional stirring, then left to stand for 18 hours. The sample was filtered with filter paper three times to separate the macerate from the pulp. The extract was then concentrated using a rotary evaporator.

Cell culture

The human breast cancer cell line T47D was cultured in RPMI with a 10% complete medium (Gibco). Medium given supplemented with 10% heat-inactivated fetal bovine serum, penicillin G and streptomycin 100 µg/ml. The cell lines were maintained at 37º C in a 5% CO_2 incubator.[18]

3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay

Cytotoxic activity was tested using an MTT assay. MTT solution of 0.5 mg/mL was pipetted 100 µL into each well. Incubate for 4 hours in 5% CO_2 incubator at 37ºC. After 4 hours, a purple precipitate of formazan crystals was observed. The condition of the cells was then assessed using an inverted microscope. The MTT reagent was removed by pipetting the supernatant, so that only a purple precipitate of formazan crystals remained. The precipitate was dissolved in each well with 100 µL DMSO. Absorbance was measured using an ELISA reader spectrophotometer at λ 550 nm.[19]

$$\% \text{ viability} = \frac{\text{absorbance of treatment} - \text{absorbance of media}}{\text{absorbance of cells} - \text{absorbance of media}} \text{ x } 100\ \%$$

Results and Discussion

Plants of the *Uncaria* genus are known to display anticancer activity.[20] There are around 38 species of the genus spread across the Asia Pacific.[21,22,23] Traditionally, one species of *Uncaria*, *Uncaria cordata* (akar kaik-kaik), is used by people in Province "Riau", Indonesia, to treat cancer. Several other species grow close to its growing location, including *Uncaria barbata* Merr, *Uncaria guianensis (Aubl.) J.F.Gmel* and *Uncaria nervosa* Elmer.

The samples used eight types, ethanolic extract of the leaves of *Uncaria barbata* (LUB), *Uncaria cordata* (Lour.) Merr (LUC), *Uncaria guianensis* (LUG), *Uncaria nervosa* (LUN) and the bark of *Uncaria barbata* (SBUB), *Uncaria cordata* (Lour) Merr (SBUC), *Uncaria guianenis* (SBUG) and *Uncaria nervosa* (SBUN) as shown in Figure 1.[24]

The findings of the phytochemical screening of the eight samples utilising the Culvenor-Fitzgerald method revealed a favourable response with Mayer's reagent.[25] The results can be seen in Table 1. The table shows the presence of alkaloid and terpenoid compounds in all the samples.

(a) (b) (c) (d)

Figure 1. (a) *Uncaria barbata*, (b) *Uncaria cordata* (Lour.) Merr, (c) *Uncaria guianensis*, (d) *Uncaria nervosa* plants

Table 1 : Phytochemical screening of the leaves and stem bark of the eight samples

Phytochemical screening	Reagen	*Uncaria barbata* (LUB)	*Uncaria cordata* (LUC)	*Uncaria guianensis* (LUG)	*Uncaria nervosa* (LUN)	*Uncaria barbata* (SBUB)	*Uncaria cordata* (SBUC)	*Uncaria guianensis* (SBUG)	*Uncaria nervosa* (SBUN)
Alkaloid	Mayer	+	+	+	+	+	+	+	+
Flavonoid	Mg	+	-	-	-	-	+	-	-
Terpenoid	LB	+	+	+	+	+	+	+	+
Steroid	LB	+	+	-	-	-	-	+	-
Phenolic	$FeCl_3$	-	-	-	-	-	-	-	-
Saponin	-	-	-	+	+	-	-	+	-

The ethanol extract was tested for cytotoxicity on T47D breast cancer cells. Breast cancer is one of the most common cancers found in women. They tested the cytotoxic effect using the MTT assay.[26-28] Six active samples against T47D cells were obtained from the test findings of the eight test. The active sample IC_{50} values are show in Figure 2 and Table 2, which indicates that the lowest IC_{50} value corresponds to the LUN sample at 64.42 µg/ml. This sample had the highest cytotoxic activity. Based on previous studies, alkaloid and terpenoid compounds have responsible in cytotoxic activity.[20] However, from the results of the phytochemical screening, all the samples contained alkaloids and terpenoids. Further research is needed to establish what compounds are present in the ethanol extract of *Uncaria nervosa* leaves (LUN). To our knowledge, there has been no reported cytotoxic activity of the ethanol extract of *Uncaria nervosa* leaves against T47D breast cancer cells. The research report that we found on the *Uncaria nervosa* plant was a cytotoxic test of the methanol extract of bark and wood. Cytotoxic test using shrimp larvae and obtained LC_{50} 1.76 and 2.66 µg/ml.[15]

The morphological profile of T47D cells after administration of *Uncaria nervosa* (LUN) leaf ethanol extract are shown in Figure 3. A concentration of 1 g/mL compared to 10 g/mL causes a large number of cells to proliferate, adhere to the bottom of the plate, grow very tightly, and be located in very close proximity to one another compared to the control, there is hardly any difference. At a concentration of 100 g/mL many T47D cells that died and were very different from the controls.

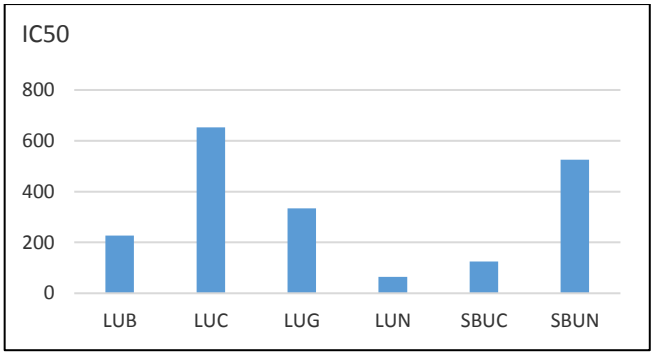

Figure 2: Comparison of samples and IC_{50} value

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Table 2: IC_{50} values of the different samples

Samples	Concentration(C)	Log C	Viability (%)	IC $_{50}$ (µg/ml)
LUB	100	2	52.007	227.30
	10	1	89.035	
	1	0	93.605	
LUC	100	2	55.838	653.13
	10	1	72.367	
	1	0	75.607	
LUG	100	2	54.400	334.65
	10	1	87.729	
	1	0	90.559	
LUN	100	2	33.145	**64.2**
	10	1	99.008	
	1	0	104.666	
SBUB				› 1000
SBUC	100	2	51.213	124.45
	10	1	69.414	
	1	0	85.165	
SBUG				› 1000
SBUN	100	2	65.132	526.02
	10	1	81.904	
	1	0	104.445	

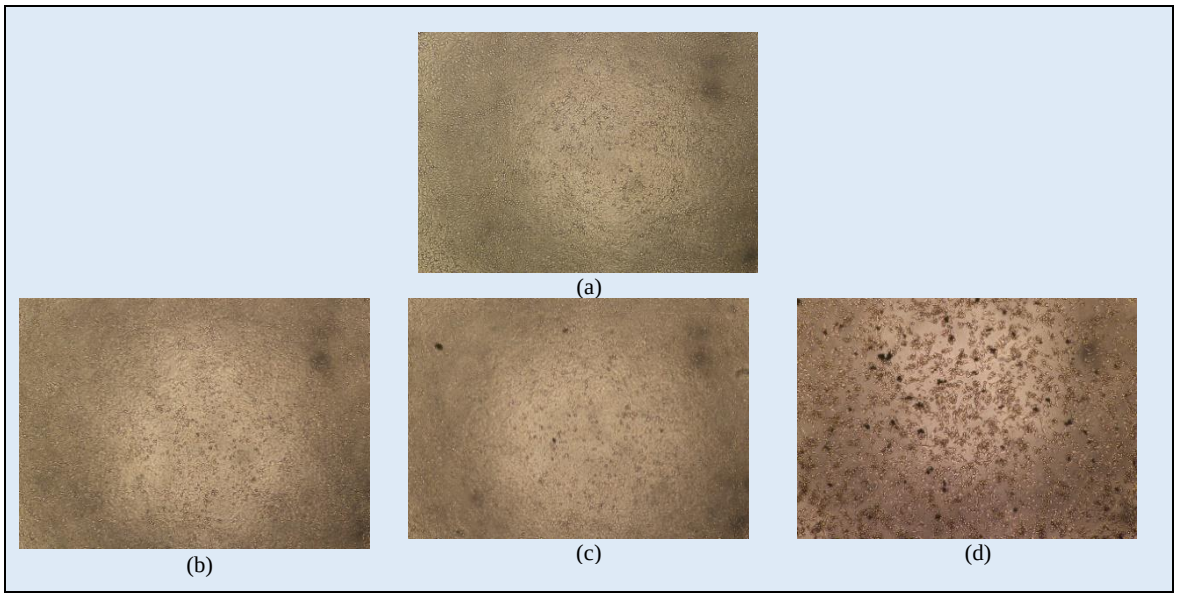

Figure 3. Morphological profile of the T47D cells after being treated with ethanol extract of *Uncaria nervosa* (LUN) leaves 1 µg/mL (b), 10 µg/mL (c) and 100 µg/mL (d) compared to control (a) for 48 h. (100 x enlarge-minute)

Conclusion

The ethanol extract of *Uncaria nervosa* leaves had a strong cytotoxic effect on T47D breast cancer cells, which was 64.42 µg/mL.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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