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Antioxidant and Anti-Breast Cancer from *Uncaria gambir Roxb* Leaves: In Silico & In Vitro Study

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ABSTRACT

Uncaria gambir Roxb (URG) is one of the plants from West Kalimantan predicted to contain antioxidant and anti-breast cancer. This study aims to test the antioxidant and anti-breast cancer potential. UGR leaves were extracted by infusion method using water for 15 minutes with 4 repetitions at a temperature 70^{o}C. A thick extract of 96.2351 grams (29.979%) was obtained from a sample weight of 321 grams of dried UGR leaves. In vitro antioxidant assay of the extract was investigated using 2,2-diphenylpicrylhydrazyl (DPPH) with positive control using quercetin and ascorbic acid, and the Ferric Reducing Antioxidant Power (FRAP) method. In silico screening showed that the biological agents in UGR had the potential as TP53 expression enhancer, antioxidant, anticarcinogenic, chemopreventive, and free radical scavenger. The antioxidant bioassay results showed IC_{50} values of 81.21 µg/mL, 73.39 µg/mL, and 9.17 µg/mL in DPPH for extract samples with positive control quercetin, sample extracts with positive control Vitamin C and Vitamin C with positive control quercetin, respectively. Meanwhile, the antioxidant activity of extract samples with the FRAP method showed value of 66,05 µg/mL. Anticancer bioassay result showed that UGR leaves extract with water solvent had the stron potentgial to inhibit 4T1 cells with IC_{50} 87.72 µg/mL.

Keywords: Anti-Breast Cancer; Antioxidant; Uncaria gambir Roxb

INTRODUCTION

The number of breast cancer sufferers in Indonesia in 2020 ranked highest (65,858 people) when compared with other cancers (WHO, 2020). In West Kalimantan, in 2013 to 2018, there was an increase in the number of breast cancer cases of 0.8/1000-1.5/1000 population (A. Iskandar et al., 2020). In general, cancer cells grew through four stages, namely initiation, promotion, progression and metastasis (Sheth & Esfandiari, 2022). If it had metastasized, breast cancer was very difficult to cure. More than 90% of cases of matastatic cancer died (Ganesh & Massagué, 2021).

An effort to prevent cancer cell growth from reaching the metastatic stage is by blocking the three stages before metastasis, which is called cancer chemoprevention. The blocking method is by administering synthetic or biological agents to delay or stop the growth of cancer cells (Steward & Brown, 2013). One biological agent that can suppress the growth of breast cancer cells is the TP53 enhancer (El-Deiry, 2023).

This agent can be obtained through synthesis or from plants. In addition, the growth of breast cancer cells can be avoided by consuming drinks that contain high antioxidants. Because cancer cells can occur due to damage to normal cells due to excessive amounts of free radicals (Tianing, 2012); (Singh et al., 2018). Providing agents can be attempted by consuming herbal tea from plants which have antioxidant potential to prevent the growth of cancer cells. Biological agents that act as antioxidants and anticancer can be predicted in silico using the PASS server (Christina et al., 2021). This software is used to predict the antioxidant and anticancer potential of compounds contained in plants (Desai & Joshi, 2019a); (Gupta et al., 2019); (Zawacka-Pankau et al., 2018).

To find out the type of plant compound, you can use the database on the website http://www.knapsackfamily.com/KNApSAcK/.
The data provided by the knapsackfamily is widely used by plant medicine researchers (Afendi et al., 2012). One of the plants from West Kalimantan which has been predicted to contain antioxidant and anti-breast cancer substances is UGR. According to Iskandar & Ramdhan (2020), this plant contains saponins, tannins and flavonoids with a total phenol content of 3.9 mg GAE/10 mg. This plant grows wild in areas that receive sufficient sunlight and high rainfall (D. Iskandar & Ramdhan, 2020). It is reported that this plant is

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efficacious as a traditional medicine and is often used by the Dayak tribe as an antimicrobial, antidiabetic, thrombolytic, antioxidant and anticancer without specifying its strength and test stages (Almeida et al., 2022).

Another study carried out by Munggari et al (2022) also stated that the UGR plant has antibacterial, anti-worm, anti-fungal, anti-inflammatory, anti-hyperglycemic, anti-hyperuricemic, anti-lipid production and anti-hyperlipidemic properties (Munggari et al., 2022). The UGR plant is one of the 327 species of traditional medicinal plants in West Kalimantan reported by Iskandar et al (2022). This plant has not been widely used, especially as a functional herbal tea drink. The part used is the leaves. This plant is believed to be a traditional anti-breast cancer medicine (D. Iskandar, Widodo, Warsito, Masruri, & Rollando, 2022). The aim of this research is to reveal the efficacy of the functional drink UGR leaf herbal tea which acts as an antioxidant and anti-breast cancer agent.

MATERIALS AND METHODS

In Silico Bioactivity Screening of Antioxidant and Anticancer Compounds in UGR Plants

Biological agents that act as antioxidants and anticancer can be predicted in silico using the PASS server (Christina et al., 2021). This software is used to predict the antioxidant and anticancer potential of compounds contained in plants (Desai & Joshi, 2019a); (Gupta et al., 2019); (Zawacka-Pankau et al., 2018). The method for screening the antioxidant and anticancer activity of compounds in UGR can use PASS online. Antioxidant and anticancer activity is indicated by a Probability to be active (Pa) value between 0 and 1 (Hartati et al., 2024); (Horn et al., 2021) (Jamuna et al., 2015); (Desai & Joshi, 2019b); (Alam et al., 2016) (Stasevych et al., 2017). The names of the compounds contained in UGR can be retrieved from the Knapsack Family database on November 20, 2023 (http://www.knapsackfamily.com/knapsack_core/result.php?sname=all&word=uncaria%20gambir). The method of searching for compound names in the date base of the knapsackfamily is considered valid (Hartono Wijaya et al., 2016).

UGR Leaf Extraction

Extraction of UGR leaves was carried out using the infusion method (boiling) for 15 minutes using distilled water as a solvent. Extraction was carried out 4 times. The boiled product is then filtered and evaporated over a water bath at a temperature of 70^{o}C until a thick extract is formed. The thick extract was evaporated using a water bath at a temperature of 50^{o}C to obtain a dry extract and then used as a sample.

Antioxidant Bioassay

DPPH Procedure

Preparation of DPPH solution

A DPPH solution was made with a concentration of 20 µg/mL in ethanol solvent

Preparation of quercetin standard solution

A quercetin solution was made with a concentration of 1000 µg/mL, then diluted to 80 µg/mL, the solvent used was ethanol

Sample solution preparation

The sample solution is made into 5 concentration points, namely 60, 70, 80, 90, 100 µg/mL for each sample, dilution using ethanol

Mixing DPPH with quercetin (as positive control)

Mix 3.8 mL of 20 µg/mL DPPH solution with 0.2 mL of 80 µg/mL quercetin then incubate (incubation time follows the optimization results for each sample)

Mixing DPPH with sample

Mix 3.8 mL of 20 µg/mL DPPH solution with samples at each concentration of 0.2 mL then incubate (incubation time follows the optimization results for each sample, in this case 20 minutes)

DPPH wavelength measurement

To find out the maximum wavelength of DPPH which will later be used for sample measurements (every time a new DPPH is made a measurement will be carried out). Measurement of DPPH absorbance at one wavelength (as a negative control). Measurement of the absorbance of the DPPH solution at one wavelength (obtained from previous measurements) which is used as negative control data

Measurement of absorbance of DPPH: Quersetin (as positive control)

Absorbance measurements were carried out using the fixed wavelength method of DPPH (the positive control was remeasured every time the DPPH was replaced because it is possible that there was a shift in the wavelength of the DPPH)

DPPH absorbance measurement Sample

Absorbance measurements were carried out using the fixed wavelength method from DPPH (D. Iskandar, Widodo, Warsito, Masruri, Rollando, et al., 2022), (D. Iskandar & Warsidah, 2020a).

FRAP Procedure

Antioxidant measurements using the FRAP method are carried out using the following procedure: preparation of vitamin C solutions with concentrations of 60, 70, 80, 90, 100 µg/mL in 1% oxalic acid solvent (as standard curve), preparation of extract sample solution, weigh 5 mg of sample and dissolve with 1% oxalic acid in a 5 mL volumetric flask (triple), each concentration of vitamin C solution and sample was added with 1 mL of 0.2 M phosphate buffer pH 6.6, then add 1 mL of 1% $K_3Fe[(CN)_6]$ solution, after that, the mixture was incubated in an oven at 50°C for 20 minutes, after incubation, 1 mL of 10% TCA solution is added, then centrifuge for 10 minutes at 3000 rpm, take 0.5 mL of the top of the solution, add 2.5 mL of distilled water, add 0.1 mL of 1% $FeCl_3$ solution, and incubate for 1 hour (Rollando et al., 2023).

Anti-Breast Cancer Bioassay

Cells were harvested at a concentration of $8x10^3$ cells/well and diluted with culture media, then planted into a 96 well microplate at 100 µL/well and incubated for 24 hours in a 5% CO_2 incubator. Before being used for treatment, the media in the plate was discarded and then washed using PBS once in the amount of 100 µL/well. Then the PBS was discarded and the test solution (1; 10; 25; 50; 75; 100; and 200 µg/mL) was given as much as 100 µL/well. The cells were then incubated again for 24 hours. After incubation, washed with PBS and added 100 µL/well of MTT reagent and incubated for 3-4 hours at 37°C. After that, 100 µL/well of stopper solution (10% SDS in 0.01 N HCl) was added and incubated overnight at room temperature (25°C) in the dark, then read with an ELISA reader at λ 595 nm and the absorbance was obtained which represents the absorbance. 4T1 cells or living vero cells. Single treatment absorbance data were converted into percent viability and used to calculate IC50 (D. Iskandar, Widodo, Warsito, Masruri, Rollando, et al., 2022); (Hariono et al., 2021).

$$\% \text{ Cell viability} = \frac{A_{sample}}{A_{control}} x100$$

The SI value can be determined by dividing the IC_{50} of Vero cells by the IC_{50} of 4T1 cells

$$SI = \frac{IC_{50} \text{ vero cell}}{IC_{50} \text{ 4T1 cell}} \text{ (Indrayanto et al., 2021)}$$

DISCUSSION

In Silico Bioactivity Screening of Antioxidant and Anticancer

Based on a search via the website http:// www.knapsackfamily.com/KNApSAcK/, this plant contains several biological agents as presented in Table I. After in silico analysis, the biological agents in UGR have the potential as TP53 expression enhancer, antioxidant, anticarcinogenic, chemopreventive, and free radical scavenger. The results of the analysis are presented in Table I.

Table I data is a reference for the next test, namely the in vitro test. In vitro test results are useful for diversifying in silico prediction data. To test antioxidant strength, It can use DPPH assay (Baliyan et al., 2022). Meanwhile, the in vitro anti-breast cancer test uses Microtetrazolium (MTT) bioassay with several cancer cells, namely MCF7, ECACC, TKR1, SKBR3 and 4T1 cells. 4T1 cells are the most widely used human breast cancer cell model, are easy to grow in the mammary glands, and metastasize to distant organs. The distribution is similar to the growth of breast cancer cells in humans (Schrörs et al., 2020). The quantity value most widely used to determine the antioxidant and anticancer potential of the breast is half maximal inhibitory concentration (IC_{50}) (Aykul & Erik, 2016).

Infusion Extraction Yield

The extraction results using an infusion procedure from 321 grams of UGR leaf powder produced 96.2351 g (29.979%) of dry extract and was then used as a sample.

Antioxidant Bioassay

The antioxidant bioassay results showed that UGR leaf extract with water solvent had radical scavenging bioactivity with a range of 66.05-81.21 µg/mL. The antioxidant bioassay results showed IC_{50} values of 81.21, 73.39, and 9.17 µg/mL in DPPH for extract samples with positive control quercetin, sample extracts with positive control Vitamin C and Vitamin C with positive control quercetin, respectively. Meanwhile, the antioxidant activity of extract samples with the FRAP method showed value of 66.05 µg/mL. Antioxidant strength can be classified into very strong (<50 µg/mL), strong between 50-150 µg/mL, weak >150 µg/mL) (D. Iskandar & Warsidah, 2020b). Based on this classification, the antioxidant bioactivity of UGR extract is a strong antioxidant

Anti-Breast Cancer Bioassay

The anticancer bioassay result showed that UGR leaves extract with water solvent had the strong potential to inhibit 4T1 cells with IC_{50} 87.72 µg/mL (Table III & Figure 1). Criteria for potential anticancer bioactivity are very strong IC_{50} <10 µg/mL, strong 10-100 µg/mL, moderate 100-500 µg/mL (D. Iskandar, Widodo, Warsito, Masruri, Rollando, et al., 2022). Weerapreyakul et al (2012)

Table I. UGR Antioxidant and Anticancer Screening Test Results (Pa)

Biological agents	*Antioxidant*		Anticancer		
	Antioxidant	*Free radical scavenger*	*TP53 expression enhancer*	*Anticarcinogenic*	*Chemopreventive*
(+) Catechin	0.810	0.842	0.959	0.795	0.788
(-) Epicatechin	0.810	0.842	0.959	0.795	0.788
(+) Epicatechin	0.810	0.842	0.959	0.795	0.788
Roxburghine B	-	-	0.731	-	-
Gallic acid	-	-	0.718	-	-
Cinchonain IA	0.803	0.716	0.857	0.702	0.720
Gambiriin C	0.803	0.798	0.954	0.757	0.830
Gambiriin A1	0.802	0.792	0.835	-	0.757
Gambiriin A3	0.802	0.808	0.800	-	0.783
Gambiriin B1	0.703	0.815	0.910	-	0.737
Gambiriin B3	0.727	0.742	0.886	-	0.755
Procyanidin B3	0.803	0.798	0.954	0.757	0.830
Procyanidin B1	0.803	0.798	0.954	0.757	0.830
Gambiriin A2	-	0.792	0.835	-	0.757
Gambiriin B2	0.703	0.815	0.910	-	0.737

Table II. Antioxidant Bioactivity of" Extract

Method	**IC_{50} (**µg/mL)
DPPH	
Extract sample with positive control quercetin	81.21
Extract sample with positive control Vitamin C	73.39
Vitamin C with quercetin as a positive control	9.17
FRAP	
Extract sample	66.05

Table III. Anticancer Bioactivity of Extract

Cell Line	**IC_{50} (µg/mL)**	**Selectivity Index (SI)**
4T1	87.72	1.24
Vero	108.90	

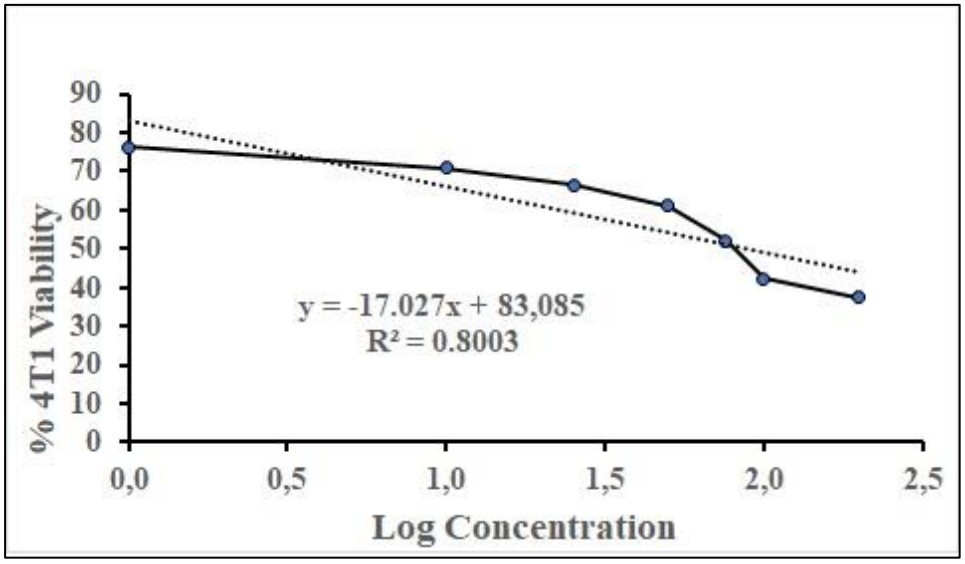

Figure 1. % 4T1 Cell Viability and Log Concentration

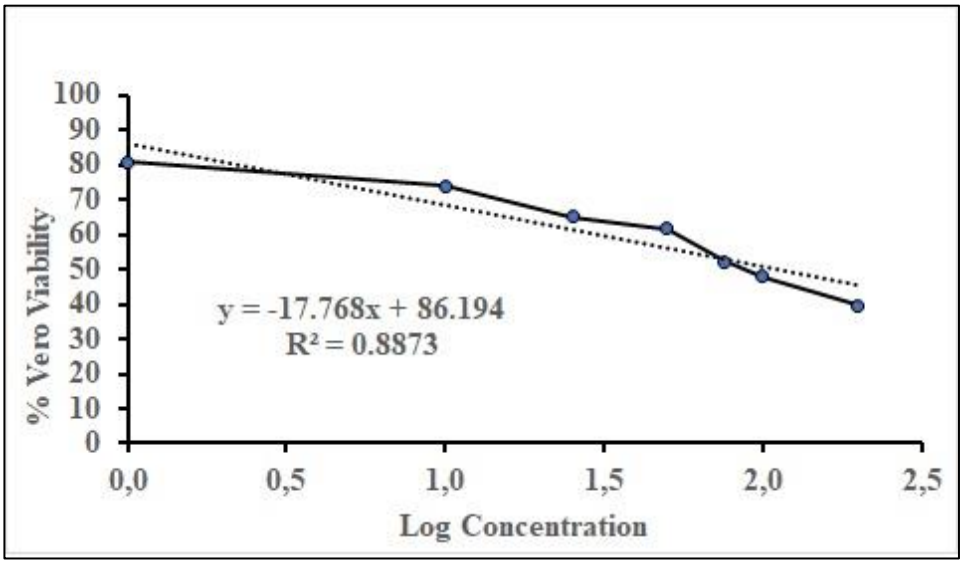

Figure 2. % Vero Cell Viability and Log Concentration

proposed an SI value lower than 3 as an anticancer candidate extract (Weerapreeyakul et al., 2012). On this basis, UGR water extract with SI 1.08 can be proposed as an anticancer herbal candidate (Table III & Figure 2).

CONCLUSION

Uncaria gambir Roxb leaves water extract can be concluded as a sample that has strong antioxidant bioactivity, strong anti-breast cancer that inhibits 4T1 cells viability, and as a selective anti-breast cancer candidate.

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