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Original Article

CYTOTOXIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF ETANOL EXTRACT OF BAJAKAH TAMPALA (*UNCARIA LANOSA VAR. FERREA* (BLUME) RIDSDALE) STEM ON BREAST CANCER CELL LINES MCF-7

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ABSTRACT

Objective: This study aimed to determine the content of phytochemical screening and cytotoxic activity indicated by the IC_{50} value of the ethanol extract of bajakah tampala stem.

Methods: Phytochemical screening for extract consists of saponins with distilled water, steroids and terpenoids were determined with the reagent glacial acetic acid and sulphuric acid, tannin using reagent 10%, $FeCl_3$, alkaloids determined with reagents Mayer, flavonoids using reagent HCl, Mg powder, and phenolic with using 2%. $FeCl_3$. The WST-8 procedures were used to investigate the cytotoxic activity of the MCF-7 breast cancer cell type.

Results: Based on the results showed that the ethanol extract of bajakah tampala stem has secondary metabolite content, namely the presence of saponins, steroids, tannins, alkaloids, flavonoids and phenolics. The results of the cytotoxic test of ethanol extract of bajakah tampala stem have cytotoxic activity with IC_{50} of 193.2 mg/ml, which is included in the moderately active category.

Conclusion: In this study, the ethanol extract of bajakah tampala stem has secondary metabolite content and cytotoxic activity against MCF-7 breast cancer cells.

Keywords: Bajakah tampala, Cytotoxic, Breast cancer, MCF-7 cells

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INTRODUCTION

Breast cancer is known to have surpassed lung cancer as the most commonly diagnosed cancer and the fifth cause of cancer deaths in the world, with an estimated 2.3 million cases and 685,000 deaths in 2020 and the cases are expected to reach 4.4 million in 2070 [1]. Unfortunately, nearly 40% of breast cancer patients develop de novo or acquired resistance after 1-3 y of tamoxifen therapy [2]. Anti-estrogen resistance is common, which ultimately leads to treatment failure, disease progression, and death [3]. This encourages various studies to find new active ingredients that are natural and safer (minimal side effects), one of which is through the search for active ingredients derived from plant parts [4].

Most (>60%) anticancer agents that have been used clinically and shown significant effectiveness are derived from plants, marine organisms, and microorganisms [5]. Compounds isolated from plants have been widely studied and show promising potential as antitumor and anticancer [6]. Phytochemical compounds such as alkaloids, flavonoids, terpenoids, polysaccharides, saponins and others have shown potential as natural bioactive compounds with strong anticancer activity [7].

Indonesia as a megabiodiversity country, has the potential to provide chemopreventive and anticancer agents because it has 6000 plant species that have been widely used by the Indonesian people as medicinal plants and herbs [8]. The search for new bioactive compounds from natural sources can be done through ethnobotanical and chemotaxonomic approaches, and one of the plants that has begun to be widely studied for bioactive compounds is bajakah tampala (*Uncaria lanosa var. ferrea* (Blume) Ridsdale) [9].

The bajakah tampala plant originates from the interior of Kalimantan Island and has not spread to other regions, including Sumatra. However, this plant is widely used empirically by rural communities in Central Kalimantan Province for various diseases. In Indonesia, especially in Central Kalimantan Province, bajakah is known as a traditional medicine by drinking boiled water from the bajakah tampala plant [10]. Bajakah (as it is called by the Dayak tribe) has become an ethnomedicine as an alternative cancer treatment, especially breast cancer [11]. There are 200 types of bajakah in Indonesia, especially in Kalimantan; although there are hundreds of types of bajakah in Indonesia, there has been no scientific research related to the cytotoxicity activity of bajakah, especially bajakah tampala bajakah species [12]. Therefore, it is necessary to explore bajakah tampala from Kalimantan, especially the cytotoxicity effect [13].

In this study, phytochemical tests will be carried out on bajakah tampala stem extract; this is due to the absence of research reports related to phytochemical compounds contained in bajakah tampala stem extract and cytotoxic activity against MCF-7 breast cancer cells. The novelty of this study is to inform the content of secondary metabolites contained in bajakah tampala stems and cytotoxic activity against MCF-7 breast cancer cells using the WST-8 method to the scientific and general public. In addition, bajakah tampala stem is expected to be used as a candidate for cancer drugs, especially breast cancer.

MATERIALS AND METHODS

Materials

The materials used in this study are bajakah tampala species obtained from the Tabakai River forest, Saka Kajang Village, Jabiren Raya District, Pulang Pisang Regency, Central Kalimantan, Cisplatin (Merck), PBS (Sigma Aldrich), DMSO (Sigma Aldrich), RPMI Media (Sigma Aldrich), WST-8 (Dojindo), and PBS (Sigma Aldrich).

Instruments

The instrument used are autoclave (Hirayama-Hiclave-HVE50), biology safety cabinet (Esco), CO_2 incubator (Thermo Scientific), inverted microscope (Zeiss), binocular microscope (Zeiss), hemocytometer (Marienfeld), centrifuge (DLab), water bath (Yihder BT-150D), micropipette (Eppendorf), microplate reader (Tecan Nano), tube (Genfollower), analytical balance (Ohaus), and glassware (Pyrex).

Determination of plants

Determination of bajakah tampala plants was carried out at the National Research and Innovation Agency (BRIN) botanical laboratory, Cibinong Bogor. The determination process is carried out using samples in the form of leaf parts complete with twigs.

Sample preparations and extractions

Samples in the form of bajakah tampala stem parts were first dried and then mashed. A total of 4000 g of dry bajakah tampala stem powder was extracted by using the maceration method using ethanol 96% solvent for 1x24 h. The maceration results obtained were then separated from the solvent using a rotary evaporator.

Phytochemical screening

Phytochemical tests were carried out with procedures that are generally carried out with the principle of color test. The alkaloid test used is Mayer. Positive samples contain alkaloids if with Mayer reagent the solution will turn into a brownish-white color. Flavonoid test using Mg powder and concentrated HCl mixed in the sample solution. The presence of flavonoid content is characterized by the formation of red, yellow or brown color accompanied by foam. Saponin test, the extract is dissolved in hot water then shaken for 30 seconds and if foam or foam is formed, it indicates the presence of saponins. Terpenoid test is done by dissolving the sample in glacial acetic acid and concentrated sulfuric acid, if the solution changes color to red or yellow, it indicates the presence of flavonoids. The steroid test is done by adding a sample solution with glacial acetic acid and concentrated sulfuric acid if the solution changes color to blue, purple or green, indicating the presence of steroids. The tannin test is done by adding a sample using 10% $FeCl_3$ and if the solution changes color to bluish-black, it shows the positive presence of tannin. As for the phenolic test is done by dissolving the extract in ethanol then added with 2% $FeCl_3$ and then shaken. If a bluish-black color is formed, it indicates the presence of phenolics in the extract.

Cytotoxic assay

In vitro cytotoxic assay was performed with WST-8 assay reagent. MCF-7 cells with a density of 8000 cells/well were distributed into 96 well plates and incubated for 24 h in culture medium. Cells were treated with bajakah tampala stem extract in cell growth medium with concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625 mg/ml and cisplatin with concentrations 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mg/ml then incubated in an incubator at 35 °C, 5% CO_2 for 24 h. After 24 h incubation, pipette WST-8 as much as 10 µl into each well, followed by incubation for 2-4 h and the final stage by reading the results with a microplate reader to get the absorbance value. For statistical analysis, the absorbance data of each well (3 replicates) was converted to the average % cell viability, then analyzed by linear regression to obtain IC_{50} using gradhpad software version 10.0.0 (131).

RESULTS AND DISCUSSION

Determination of plants

Bajakah tampala taken from the Tabakai River forest, Saka Kajang Village, Jabiren Raya District, Pulang Pisang Regency, Central Kalimantan was determined to determine the type or species of bajakah tampala plants carried out at BRIN while the results of determinations that have been carried out have the species name Uncaria lanosa var. ferrea (Blume) Ridsdale with number of letter determination is B-493/II.6.2/IR.01.02/3/2023.

Extraction

The filtrate obtained from the maceration of bajakah tampala stem powder using 96% ethanol solvent is then separated from the solvent using a rotary evaporator. The resulting thick extract of bajakah tampala stem is blackish red in color with a distinctive aroma of bajakah tampala stem weighing 693. 44 g, as shown in the table 1.

Phytochemical screening

The results of the phytochemical test on bajakah tampala stem extract showed the presence of metabolite secondary compounds as shown in the table 2.

Cytotoxic assay

Bajakah tampala stem has been extracted from Central Kalimantan shows cytotoxic activity included in the moderately active category with an IC_{50} value of 193.2 mg/ml while the IC_{50} value of cisplatin as positive control is 21.96 mg/ml as attached in the table 3.

Table 1: Percent yield

Extract type	Organoleptic	Simplisia weight	Extract weight	% Yield
Concentrated extract	Blackish-brown colour with a distinctive aroma of bajakah tampala	4000 g	693.44 g	17.36

Table 2: Metabolite secondary of bajakah tampala stem

No	Secondary metabolite	Extract of bajakah tampala stem
1	Saponins	+
2	Steroids	+
3	Terpenoids	–
4	Tannin	+
5	Alkaloids	+
6	Flavonoids	+
7	Phenolics	+

Remarks: (+) Positive for containing secondary metabolites, (-) Negative for not containing secondary metabolites

Table 3: Values IC_{50} of bajakah tampala stem extract vs cisplatin

Sample	Concentration (mg/ml)	Survival rate			Survival rate (%)	IC_{50} (mg/ml)
		1	2	3		
Tampala	1000	4.01	2.10	6.48	4.20±2.19	
Bajakah Stem	500	15.14	15.16	16.48	15.59±0.76	193.2
Extract	250	51.24	47.52	49.10	49.28±1.87	
	125	92.07	87.13	70.73	83.31±11.17	
	62.5	91.62	94.83	89.34	91.93±2.75	
	31.25	124.15	120.68	102.99	115.94±11.35	
Cisplatin	50	7.71	7.09	7.22	15.77±0.32	
	25	30.53	34.50	37.51	42.61±3.50	21.96
	12.5	32.99	36.73	41.01	45.34±4.01	
	6.25	39.97	42.35	51.03	52.89±5.82	
	3.12	52.17	50.41	55.56	61.15±2.61	
	1.56	55.92	58.22	59.76	66.41±1.93	

Meanwhile, to see the relationship between concentration and survival rate based on calculations using the graphpad application can be seen in the fig. 1. fig. 1 showed that with increasing concentration of ethanol extract of bajakah tampala, the percent survival rate is getting smaller and vice versa that the concentration of bajakah tampala extract is small, the percent survival rate is getting bigger.

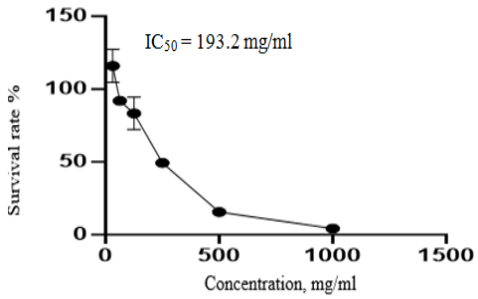

Fig. 1: Graph of the relationship between extract concentration and survival rate

DISCUSSION

The stem of bajakah tampala (*Uncaria lanosa var. ferrea* (Blume) Ridsdale) was used in this study, and it was specifically obtained from the Tabakai River forest, Saka Kajang Village, Jabiren Raya District, Pulang Pisang Regency, Central Kalimantan. The botanical laboratory of the National Research and Innovation Agency (BRIN) Cibinong, Bogor Regency, is used to determine bajakah tampala plants with the goal of ensuring the correct identity of the plant species used by adjusting the morphological characteristics of bajakah tampala plants with existing literature or literature to avoid errors in plant collection [14]. The determination results are in accordance with the observations of bajakah tampala plant stems compared to the plant collection found in the BRIN botanical laboratory so that it is stated that the sample is a plant with the species *Uncaria lanosa var. ferrea* (Blume) Ridsdale, which belongs to the family *Rubiaceae.*

The samples were extracted using the maceration procedure, which requires simple instruments and avoids heating, which might disrupt the chemical compound arrangement. The extract yield is the percentage of the final weight of the extract to the initial weight of the extract. The number of bioactive components present in a high degree of yield is indicated by the value.

The presence of secondary metabolite content in the form of saponins, steroids, tannins, alkaloids, flavonoids, and phenolics is shown in table 1 based on the results of phytochemical screening in 96% ethanol extract of bajakah tampala stems, but not terpenoid compounds because terpenoid compounds have a cyclic structure in the form of alcohol which causes these compounds to tend to be semipolar so that their bond with polar ethanol solvents is very weak [15].

In this work, anticancer activity was tested *in vitro* using MCF-7 cancer cells. MCF-7 cancer cells have estrogen receptors (ER+), overexpression of Bcl-2, resistance to chemotherapeutic agents due to over-expression of PgP (P-glycoprotein), and MCF-7 cells require ATP to push drugs out of cells, so the concentration of chemotherapy drugs in cells decreases as chemotherapy effectiveness decreases [16].

The WST-8 method is used in cytotoxic testing to determine cell viability and proliferation, in which WST-8 tetrazolium salts are reduced in cells by dehydrogenase enzymes to orange formazan products that dissolve in tissue culture media. The amount of formazan produced is proportional to the number of live cells, and the absorbance is quantified with a microplate reader [17]. Based on the calculated IC_{50} value of 193.2 mg/ml, ethanol extract of bajakah tampala stem exhibits moderately active cytotoxic action on MCF-7 cell line. The IC_{50} value of cisplatin, employed as a positive control in this investigation, was 21.96 mg/ml, indicating that cisplatin had cytotoxic activity when compared to bajakah tampala stem extract. Cisplatin is a highly potent cytotoxic agent that acts non-selectively by causing toxicity to both cancer cells and normal cells, particularly the spinal cord, which is a normal cell with a high proliferation rate [18].

The cytotoxic activity test revealed a lower percentage of cell viability when the concentration series of bajakah tampala stem extract was added, as illustrated in fig. 1 [19]. The IC_{50} value is the magnitude of the concentration of ethanol extract of bajakah tampala stem that can inhibit 50% of MCF-7 cancer cell growth, with anticancer activity defined as IC_{50} 10 mg/ml (very strong cytotoxicity), IC_{50} 10-100 mg/ml (strong cytotoxicity), and IC_{50} 100-500 mg/ml (moderate cytotoxicity) [20].

The flavonoids content of the ethanol extract of bajakah tampala stem contributes to its cytotoxic activity, which inhibits the proliferation of MCF-7 cancer cells by inhibiting protein kinase, thereby inhibiting the signal transduction pathway from the cell membrane to the cell nucleus [21]. Alkaloids chemicals in ethanol extract of bajakah tampala stem have an anticancer mechanism of action by triggering apoptosis in cancer cells, allowing the cell division process to be controlled [22]. Steroids substances, on the other hand, have the ability to stop the cell cycle in the G2/M phase by stabilizing the sponde threads in the mitotic phase [23].

According to the findings, the ethanol extract of bajakah tampala stem has potential cytotoxic activity with an IC_{50} value of 193.2 ppm, which falls into the moderate category, but more research is needed to identify the active compound groups and molecular structures that are cytotoxic so that they can be developed into cancer drug candidates, particularly for breast cancer.

CONCLUSION

Ethanol extract of bajakah tampala stem based on the results of phytochemical screening contains saponins, steroids, tannins, alkaloids, flavonoids and phenolic compounds. As for the bajakah tampala stem extract, it has cytotoxic potential against MCF-7 breast cancer cells and this is evidenced by the IC_{50} value of 193.2 mg/ml, which is included in the moderately active category.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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