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Cytotoxicity of Kamandin Saebo *Glossocardia leschenaultii* [Cass.] Veldkamp Extract with Various Solvents on T47D Cells

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Abstract

In Indonesia, cancer is one of the diseases with a high mortality rate. In 2018, there were 348,809 cases of cancer, with 16.7% of all cases being breast cancer. In Indonesia, breast and cervical cancer are the most common types. The purpose of this study was to determine the secondary metabolite content of compounds and the anticancer activity of kamandin saebo extract against T47D breast cancer cells from kamandin saebo (*Glossocardia leschenaultii* [Cass.] Veldkamp) samples using various types of solvents. The methods used were sample preparation, moisture content analysis, extraction using the ultrasonic method, secondary metabolite analysis using reagents, and anticancer testing against T47D breast cancer cells. The plants were washed thoroughly to obtain powder and sieved with a 60 mesh, with a moisture content of 11.5% (w/w), and the extraction results obtained concentrated extracts from various types of solvents. The secondary metabolite content of kamandin saebo is flavonoids, steroids, alkaloids, and tannins. The anticancer activity of methanol, ethyl acetate, and n-hexane extracts, with IC_{50} values of 340, 272, and 107 µg/ml, respectively. The n-hexane extract has anticancer potential compared to ethyl acetate and methanol extracts.

Keywords: Glossocardia leschenaultii [Cass.] Veldkamp; Anticancer; Ultrasonic; IC_{50}

INTRODUCTION

In Indonesia, cancer is one of the diseases with a high mortality rate. In 2018, there were 348,809 cases of cancer, with 16.7% of all cases being breast cancer. In Indonesia, breast and cervical cancer are the most common (WHO 2020). Uncontrolled cell growth in a part of the body is known as cancer, which causes a decline in the function of organs, organ systems, and the body itself (American Cancer Society, 2019).

The incidence of breast cancer has continued to increase since 1980. Many cases of breast cancer are found in the early stages, when the tumor is still small and has not spread. Age and gender are two factors closely related to the risk of breast cancer. Women have a higher risk of developing breast cancer, and the risk increases with age. Breast cancer is generally treated with chemotherapy. However, side effects such as nausea, vomiting, peripheral neuropathy, and bone marrow suppression can occur due to long-term use of chemotherapy (Joseph T. DiPiro, 2020).

The development of plant-based drugs has been the subject of extensive research recently (Amin et al., 2025; Imaniar et al., 2022), to minimize side effects compared to synthetic drugs. Vincristine and vinblastine are examples of cancer drugs derived from medicinal plants (Mekky et al., 2018; Dhyani et al., 2022). Both drugs are effective in inhibiting the growth of breast cancer cells (Dowd et al., 2017). Efforts to develop effective anticancer drugs require a scientific understanding of how medicinal plants function as anticancer agents.

One plant with purported anticancer effects is Kamandin Saebo (*Glossocardia leschenaultii* (Cass.) Veldkamp). This plant has been used by the Madurese people to treat stroke, cancer, and paralysis (Purwanti et al., 2023). Research on *Glossocardia bosvallea* (L.F.) DC methanol extract has shown significant antibacterial activity against Staphylococcus aureus s (32±1.6) (Ramakrishnan et al., 2014). The n- hexane extract of *Glossocardia bosvallea* (L.F) DC was also tested for antioxidant activity with an IC_{50} of

31.55±1.45 μg mL^{-1} (Rajopadhye and Upadhye, 2012). The phytochemical content of Glossocardia bosvallea (L.f.) DC is phenol, quinone, flavonoid, tannin, terpenoid, and alkaloid (Kanchan, 2021). The chemical compounds successfully isolated from Glossocardia bosolia (L.F) DC are terpenes (1S)-(-) β-pinene, (-)-sabinene, and (4S)-(-)-limonene (1) (Darshani, 2023).

Research on the cytotoxic test of Kamandin Saebo *Glossocardia leschenaultii* (Cass.) Veldkamp extract on T47D cells using the MTT method has never been done before, and there are very few publications on this topic. To date, this plant has only been reported in relation to its morphology and its use by the Madurese community. One of the uses of this plant is for stomachaches, headaches, and cancer (Hariri et al., 2021; Purwanti et al., 2023). Therefore, the researchers were interested in further investigating this plant, particularly in relation to breast cancer/T47D cells. The objective of this study is to determine the secondary metabolite profile and cytotoxic activity of Kamandin Saebo extract against T47D breast cancer cells.

METHODOLOGY

Materials and Instrumentals

The equipment used in this study included glassware, an oven, a simple distillation apparatus, a rotary vacuum evaporator, a micropipette, an Ultrasonic LC60 H (Elmer), a laminar flow air, a microscope, a CO_2 incubator, and an ELISA reader to read the absorbance of cytotoxic test cells.

The materials used were [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] (MTT) (Sigma Chemical, St. Louis, MO), distilled water, methanol, ethyl acetate, n hexane, Mg powder, HCl, Liebermann-Burchard, Mayer, Wagner, ferric chloride, dichloromethane p.a., SDS 10%, trypsin, MK RPMI, and alcohol.

Methods

Sample preparation

Kamandin saebo plants were weighed, washed with water, and cut into small pieces. The samples were dried by air. The samples were blended into powder and sieved with a 60 mesh sieve.

Moisture Content Analysis

Water content determination refers to the study (Fitri et al., 2023). Weigh 2 grams of plant powder and place it in a previously dried porcelain dish. After that, the sample is dried in an oven at 105°C for 3-4 hours or until a constant weight is achieved. After drying, the sample is cooled in a desiccator for more than or less than 15 minutes before being weighed again. The difference in weight before and after drying is used to calculate the moisture content.

Preparation of Kamandin Saebo Extract

One gram of sample is placed in an Erlenmeyer flask, dissolved in 10 mL of methanol, and extracted using an ultrasonic device (frequency 20–40 kHz) for 30 minutes at a temperature of 40°C. The same treatment is applied to n-hexane and ethyl acetate solvents. The extraction results are filtered with filter paper, and the solvent is evaporated with a vacuum rotary evaporator, and the yield is calculated.

Phytochemical Test

Phytochemical testing was performed by adding reagents or other elements, including tests for alkaloids, flavonoids, steroids, and tannins, based on the studies by Hasanela et al. (2023) and Harborne (1987).

Cytotoxicity Test

Cytotoxicity testing of methanol, ethyl acetate, and n-hexane extracts of kamandin saebo against P388 murine leukemia cells using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method in vitro (Alley et al., 1988; Fajar Aldin et al., 2019; Hasanah et al., 2019). Test solutions with varying concentrations (31, 63, 125, and 250 μg/ml) were added to the P388 murine leukemia cell culture and incubated in 5% CO_2 for 4 hours at 37°C. Cell growth activity after treatment was measured by adding MTT salt indicator to produce purple formazan crystals. After 4 hours, a stop solution was added to the test cells to dissolve the formazan crystals. Cell cytotoxic activity and IC_{50} determination ofthe test samples were performed using an ELISA reader at a wavelength (λ) of 540 nm.

Data Analysis

The IC_{50} value was analyzed using linear regression with Microsoft Excel concentration and cell viability percentage.

RESULTS AND DISCUSSION

Moisture Content Analysis

The results of the moisture content analysis of the Kamandin Saebo sample using the gravimetric method were 1.516 (w/w). This moisture content value is in accordance with (Fitri et al., 2023), which states that the standard moisture content for powdered herbal medicine is < 10%. The purpose of moisture content analysis is to determine the shelf life of the sample and to slow down fungal growth.

Kamandin saebo extraction

The Kamandin saebo sample, in the form of dried and pre-prepared powder, was extracted using the ultrasonic method. The basic principle of ultrasonics is the transfer of ultrasonic waves into a particle, which increases mechanical stress and causes a cavitation effect. The solvent can diffuse and break down the cell walls, and the active compounds can be extracted due to the cavitation effect. One of the advantages of ultrasonic extraction is that it can be processed quickly, increases yield, and requires little solvent (Sholihah et al., 2017). Solvents used for extraction with different polarities are methanol, ethyl acetate, and n-hexane. The extraction filtrate was filtered with filter paper, each extract was concentrated with a vacuum rotary evaporator, and the yield was calculated. The methanol extract was evaporated to obtain polar metabolites. The ethyl acetate extract was used to extract semi-polar compounds. Similarly, the n hexane extract was used to obtain non-polar compounds.

Table 1. Yield results with various solvents

Solvent	Percentage (%)
Methanol	0.6790
Ethyl acetate	0.7152
N-hexane	0.3064

Phytochemical Test

Phytochemical testing is a qualitative analysis to determine the content of secondary metabolites in an extract (Masoko, 2017). Samples of Kamandin saebo ethyl acetate extract were placed in a drop plate and then reagents were added according to the identified compounds. The following are the results of the phytochemical testing of Kamandin saebo ethyl acetate extract (Table 2).

Table 2. Results of phytochemical testing of Kamandin Saebo with reagents

Test	Testing Methods	Observation	Results
Flavonoids	HCl and Mg powder	 Dark-red	++
Steroid	Lieberman Burchard	 Blue-green	++
Tannin	$FeCl_3$	 black	+++
Alkaloid	- Dragrendrof	 Orange	++
	- Wagner	 Reddish brown	+

Cytotoxicity test

Wagner Reddish brown + The cytotoxicity test of methanol, ethyl acetate, and n-hexane extracts of kamandin saebo was conducted on T47D cells as a breast cancer model using the MTT method. The concentrations of the extracts used were 31, 63, 125, and 250 µg/ml, respectively. Cell viability was tested using MTT reagent after 24 hours of incubation in a CO_2 incubator (Hasanah et al., 2025). Figure 1 shows the effect of concentration on cell viability percentage. The n-hexane extract was able to reduce viability percentage better than the ethyl acetate and methanol extracts.

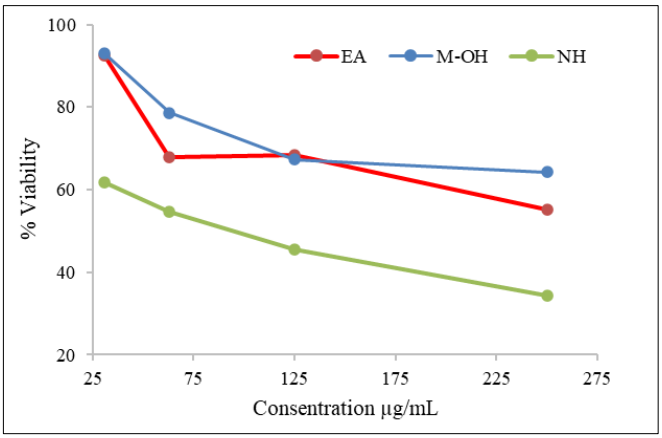

Figure 1. Relationship Between the Concentration of Each Extract and the Percentage of Cell Viability

The graph above shows that n-hexane extract has better potential than ethyl acetate and methanol extracts. Based on the results of secondary metabolite testing, Kamadin saebo contains compounds

including flavonoids and steroids that have potential as anticancer agents. *G. leschenaultii* (Cass.) Veldkamp has a flavonoid content of 39.76543 mg QE/g (Zahro, 2024). The content of active compounds, such as phenolic compounds, plays a role in activity testing (Mahardika, 2023; Purwaningsih, 2023). The antioxidant activity of the ethyl acetate extract of Kamandin saebo was also tested using the DPPH method, with an IC50value of 222.460 ppm (Shafia, 2023).

G. bosvallea (L.F) DC is another Glossocardia species that has also been tested for antioxidant activity with an IC50of 31.55±1.45 µg mL-1 (Rajopadhye and Upadhye, 2012). Secondary metabolites resulting from the isolation of terpenoid compounds from G. bosolia (L.F) DC are (1S)-(-)-β pinene, (-)-sabinene, and (4S)-(-)-limonene (1) (Darshani et al., 2023). The methanol extract of *G. bosvallea* (L.F.) DC has significant antibacterial activity against Staphylococcus aureus (32±1.6) (Ramakrishnan R et al., 2014).

Based on the IC50value, the cytotoxicity of an extract is classified into three categories: potential (IC_{50}< 100 µg/ml), moderate (IC_{50}< 1000 µg/ml), and low (IC_{50}> 1000 µg/ml) (Prayong, Barusrux, and Weerapreeyakul 2008). Based on Figure 2, the n-hexane extract shows moderate activity (IC50 = 109 µg/ml), followed by ethyl acetate (IC50 = 272 µg/ml) and methanol (IC50 = 340 µg/ml).

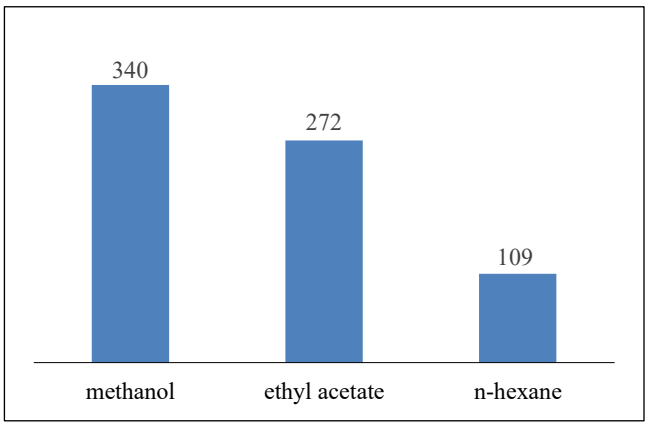

Figure 2. IC_{50} values of methanol, ethyl acetate, and n-hexane extracts

Morphological observations of cancer cells were conducted using an inverted microscope for each test extract. Observations on the test cells showed that dead cells had a clear, round shape, while living cells were elongated. The results of observations for each extract on the test cells are shown in Figure 3.

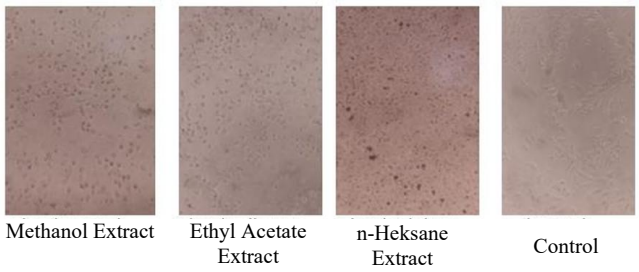

Figure 3. Morphological observations of cancer cells

CONCLUSION

Kamandin saebo Glossocardia leschenaultii (Cass.) Veldkamp was extracted using ultrasonication with three solvents: methanol, ethyl acetate, and n-hexane. They were then tested for cytotoxicity using the MTT method. The cytotoxicity test values expressed as IC_{50} for the methanol, ethyl acetate, and n-hexane extracts were 340, 272, and 109 (µg/ml), respectively.

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