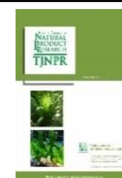
Trop J Nat Prod Res, February 2025; 9(2): 756 - 764 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org



Original Research Article

Anticancer Activity of Leaf Extracts and Fractions of *Etlingera elatior* (Jack) R.M.Sm. from North Luwu, Indonesia, using the MTT Assay Protocol

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ARTICLE INFO

Article history:
Received 13 September 2024
Revised 19 September 2024
Accepted 02 February 2025
Published online 01 March 2025

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ABSTRACT

Cancer is a disease caused by abnormal and uncontrolled cell growth. *Etlingera elatior* (Jack R.M. Smith is known to contain several compounds, one of which is flavonoids reported to have strong anticancer activity against various cancer cells. This study aims to test the anticancer activity of extracts and fractions of *E. elatior* leaves from North Luwu on several cell lines using the MTT assay protocol. Extracts were obtained from maceration using 70% ethanol and the crude extract obtained was fractionated using the liquid-liquid extraction method with n-hexane ethyl acetate and water. The anticancer activity of the extracts and fractions was evaluated using the MTT method. The results of anticancer activity of Patikala leaf extract from North Luwu against WiDr, MCF-7, HeLa and Vero cells were 67.5 µg/mL, 213.5 µg/mL, 263.4 µg/mL and 592 µg/mL, respectively. The three fractions (n-hexane, ethyl acetate and water) also demonstrate activity against WiDr and Vero cell lines with the inhibitory potential of 214.7 µg/mL, 49.9 µg/mL, and 369.3 µg/mL, respectively, while against Vero cells the activity was 1626.05 µg/mL 546.8 µg/mL, and 7574.24 µg/mL, respectively. The study concluded that patikala leaf extract showed moderate to weak anticancer activity against several cell lines, with selective activity against WiDr cells but was not cytotoxic to Vero cell lines. The extract and fractions showed anticancer activities against all the cell lines used, but the ethyl acetate fraction was the most selective exhibiting a moderate cytotoxic. Meanwhile, all the fractions were not toxic to Vero cells.

Keywords: *Etlingera elatior*, WiDr cell, MCF-7 cell, HeLa cell, Vero cell

Introduction

Cancer is one of the major causes of death worldwide. In 2015, cancer accounted for 8.8 million, or 22%, of all non-communicable illness deaths[1]. Anomalies like cancer are defined by aberrant cell proliferation. According to data from Globocan (Global Burden Cancer), there were 18.1 million new cases and 9.6 million deaths in 2018. Yogyakarta has the highest frequency at 4.86 per 1000 people, followed by West Sumatra at 2.47 per 1000 and Gorontalo at 2.44 per 1000. Some types of cancer that cause the most deaths in the world are breast cancer, cervical cancer and HeLa cancer. To counteract cell oxidation, antioxidants are needed which function to stabilise cells by donating electrons so that cells are protected from these reactive compounds (free radicals).[2]One of the treatments for cancer is chemotherapy. Chemotherapy is used primarily to kill cancer cells and inhibit their development. However, chemotherapy's side effects result from the medications inability to differentiate between cancer cells that grow excessively and healthy cells that ordinarily increase quickly as well. Therefore, natural ingredients have the potential as an alternative for cancer treatment because they have lower side effects compared to chemotherapy.[3]

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Citation: Utami YP, Yulianty R, Djabir YY, Alam G. Anticancer Activity of Leaf Extracts and Fractions of *Etlingera elatior* (Jack) R.M.Sm. from North Luwu, Indonesia, using the MTT Assay Protocol. Trop J Nat Prod Res. 2025; 9(2): 756 – 764 https://doi.org/10.26538/tjnpr/v9i2.44

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Traditional medicine is believed to have properties that can cure diseases that are difficult to cure. Some of the causes of diseases such as cancer, inflammation, atherosclerosis, and premature ageing are free radicals such as hydrogen peroxide, superoxide, hydroxyl radicals, and other radical chemicals often referred to as reactive oxygen species (ROS). These reactive oxygen and radical compounds oxidise the cells of the human body disrupting the growth of cells and causing disease.[2]
Since ancient times, people have utilised medicinal plants to cure a variety of illnesses, including cancer. The huge diversity of plants is a potential source of natural ingredients/chemical compounds that have antitumor and cytotoxic activity. Many studies report the relationship between consuming medicinal plants and carcinogenicity. The content of various phytochemical compounds in medicinal plants has been shown to treat cancer at the initiation, promotion and progression stages.[4]

Patikala leaves are known to contain flavonoids, polyphenols, saponins, tannins and steroid compounds that function as antioxidants.[5] In addition, the antioxidant potential of patikala leaves using several methods, namely DPPH, ABTS and FRAP is 42.45 ± 1.37 µg/mL, 26.46 ± 0.09 µg/mL, and 78.52 ± 0.26 mgAAE/g extract has shown potential as an anticancer medical herbal.[6] The potential of anticancer activity of natural materials can be done using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colourimetric assay by reading the absorbance of formazan produced using an ELISA reader, with the results expressed as half maximal inhibitory concentration (IC_{50}) value of the test agent.[7] Thus, research was conducted to evaluate the anticancer activity of extracts and fractions of patikala leaves from North Luwu against several cell lines using the MTT method.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Materials

The materials used in the study include Stirring rod, Biosafety Cabinet (BSC), porcelain cup, conical flasks (Corming®), separatory funnel (Pyrex®), desiccator, ELISA reader (Thermo Fischer Scientific®), 250 mL Erlenmeyer (Pyrex®), 100 mL and 250 mL beakers (Pyrex®), 10 mL, 100 mL measuring cylinders (Iwaki®), CO_2 incubator, watch glass, filter paper, laminar airflow (Esc®), drying cabinet, fume hood, GF 254 KLT plates, 96-well microplate (Iwaki®), micropipette (Bio Rad®), inverted microscope (Olimpus®), analytical balance, capillary tube, small tube rack, horn spoon, stative and clamp, Eppendoft tube, (Nesco®), small test tube and vacuum rotary evaporator (Buchi®). Patikala Leave Extract, FBS (Fetal Bovine Serum) (GIBCO), $FeCl_3$ (Iron III Chloride), Fungizone (GIBCO), HCl, label paper, Rose Park Memorial Institute 1640 (RPMI 1640) medium (GIBCO), 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) MTT reagent, ethyl acetate, n-Hexan, Pen-Strep (GIBCO), PBS (Phosphate buffered saline) (HImedia), $AlCl_3$ (Aluminum chloride), SDS stopper, silica gel, Trypsin EDTA (GIBCO).

Cell lines

These include WiDr, HeLa, MCF-7 and Vero cells obtained from cancer cell stocks at the Cell Culture Laboratory of Muhammadiyah University of Yogyakarta.

Plant collection, Identification and Drying

Fresh leaves of *Etlingera elatior* (Jack) R.M. Smith were collected from Tallu Tulak Town, Sabbang Prefecture, North Luwu Locale, and South Sulawesi (2°36'25"S 120°08'30"E) in 2023. The plant sample was identified and verified at the Plant Assurance Unit, Pharmacognosy-Phytochemistry Research Facility, Staff of Drug Shop, Muslim College, Indonesia where a voucher number 0064/C/UD-FF/UMI/IX/2023 was assigned and the herbarium sample was deposited.

The plant samples were washed with clean water to remove dirt and other debasements, and sorted while they were still moist. The samples were then broken into smaller pieces and rinsed under running water. The chopped leaves of patikala were air-dried for a week, sorted and ground to powder.

Sample preparation

The powdered leaf sample was macerated with % ethanol, filtered using Whatman No. 1 filter paper and the filtrate was evaporated to dryness using a rotary evaporator at 40°C. The crude ethanol sample was fractionated in a separating funnel using 50 mL x 3 of n-hexane to obtain the n-hexane fraction. Also, 10 grams of thick ethanol extract from patikala leaf was dissolved with 50 mL of distilled water and partitioned in 50 mL x 3 of ethyl acetate to give the ethyl acetate fraction. The residual fraction is evaporated to dryness to obtain the water fraction.

Anticancer Activity of Extracts and Fractions on WiDr, HeLa, MCF-7 and Vero cells

The culture medium consists of a mixture of RPMI (Roswell Park Memorial Institute) media with Penicllin-Streptomycin 1.5%, Fungizone 0.25% and FBS (Fetal Bovine Serum) 10% so that it becomes complete media[8].

Cultivation and Harvesting of Cancer Cells

WiDr, HeLa, MCF-7 and Vero cells were obtained from the Cell Culture Laboratory of the Cell Culture Laboratory of Muhammadiyah University of Yogyakarta. After being removed from the CO_2 incubator, the cells were examined to determine their state (till 80% of confluent cells were ready to be harvested). Cell harvesting followed the established Protocol. Then the cells were counted according to the Cell Counting Protocol (10,000 per well in 96 well plates or adjusted to the type of cells used). Cells were plated as much as 100 µL in each well. Three empty wells were left for media control. The cell distribution was observed with an inverted microscope and documented. The cells were incubated for a day at 80% confluency, meaning that they were already attached and prepared for treatment.[8]

Preparation of Test Solution

Extract and fraction stocks were prepared at 10 mg/1 mL DMSO and then diluted to a concentration of 1000 ppm by 100 µL in 1 mL DMSO.

Cell treatment with test samples

Before treatment, the condition of the cells that had been distributed in the wells was first observed, and then appropriate sample concentrations for treatment were made. Specifically for WiDr, HeLa, and MCF-7 cells, the concentration series were 31.5 µg/mL, 62.5 µg/mL, 125 µg/mL, and 250 µg/mL, while for Vero cells the concentrations were 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL. The plates containing cells and media were removed from the CO_2 incubator into the BSC (Biosafety Cabinet). Then the cell media was discarded by reversing the plate 180° above the waste with a distance of ± 10 cm, then the remaining liquid in the plate was drained with tissue pressed on the plate. After which, the cells were washed with PBS (Phosphate buffered salina) as much as 100 µl for each well, and then the PBS was discarded as before. The cells were treated with the sample concentration series in triplicates. After that, the plates were incubated in a CO_2 incubator for 24 hours, or 48 hours, depending on the sample effect on the cells.[8]

Activity Test with MTT Assay Method

After the cell incubation period, the cells were removed from the CO_2 incubator and documented. Furthermore, for a single well plate, MTT reagent was introduced at a concentration of 0.5 mg/mL. The samples were rinsed with PBS and the wells were filled with 100 µL of MTT reagent (including medium control). The wells were incubated for another 2-4 hours and the cells were examined with an inverted microscope. A stopper containing 100 µL of 10% SDS in 0.01 N HCl was added in case formazan developed. After covering the plates with foil, they were left to incubate at room temperature for the entire night. Then the plates were opened and read with ELISA'S reader at λ = 550-620 nm.[8]

Computation of percentage cell inhibition (IC_{50} value)

The percentage of viable cells was calculated using the activity test findings, which were expressed as absorbance response (uptake) using the equation below.[9]

$$\% \text{ Viable cells} = \frac{\text{Treatment absorbance} - \text{Media control absorbance}}{\text{Cell control absorbance} - \text{Media control absorbance}} \times 100\%$$

The acquired data were plotted, and the IC_{50} value and percentage of surviving cells were computed from a linear regression equation of the percentage of live cell viability against log concentration using Microsoft Excel so that the equation $y = bx + a$. The IC_{50} value is the concentration that inhibited 50% of cancer cell proliferation, which also suggests a compound's possible cell cytotoxicity. The U.S. National Cancer Institute (NCI) States categorised the IC_{50} harmful chemicals into four groups: high ($IC_{50} < 20$ µg/mL), medium (IC_{50} 21-200 µg/mL), weak (IC_{50} 201-500 µg/mL), and non-toxic ($IC_{50} > 501$ µg/mL).

Statistical analysis

Results were computed in triplicates and expressed as mean ± SD using the Microsoft Excel Statistical package 365, 2022. For every analysis, a p-value of less than 0.05 was deemed statistically significant.

Results and Discussion

The activity of patikala leaf extracts and fractions (*Etlingera elatior* (Jack) R.M. Smith) against WiDr cancer cells was evaluated *in vitro* using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and results were expressed in IC_{50} values. The extraction process was based on the polarity of the solvents used, from the non-polar hexane to the most polar ethanol. The type of

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 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

extracting solvent used influences the concentration of active chemicals in the extract. This investigation employed n-hexane, ethyl acetate and ethanol as the solvents of extraction. The extract's anticancer potential was evaluated using WiDr, HeLa, MCF-7, and vero cell lines (Table 1). The anticancer evaluation utilised the MTT method because it has various advantages including fast, sensitive, and accurate and many samples can be tested using the MTT method using the IC_{50} value as a parameter.

Table 1: Cancer cells Absorbance Result of ethanol extract of patikala leaf (*Etlingera elatior* (Jack) R.M.Smith) of North Luwu origin

No.	Cancer Cell	Concentration (µg/mL)	Absorbance Mean±SD
1	WiDr	250	0.073±0.001
		125	0.166± 0.018
		62.5	0.586±0.028
		31.25	0.78±0.038
2	MCF-7	1000	0.072± 0.002
		500	0.080±0.002
		125	0.446±0.009
		62.5	0.538±0.007
3	HeLa	1000	0.111±0.002
		500	0.166±0.008
		250	0.465±0.014
		125	0.559 ±0.029
4	Vero	1000	0.203± 0.004
		500	0.345±0.017
		250	0.381±0.022
		125	0.412±0.003

Table 2: Cell Control and Media Control Absorbance of ethanol extract of Patikala Leaf Against Cancer Cells of North Luwu Origin

No	Cancer Cell	Treatment	Mean±SD
1.	WiDr	Absorbance of cell control	0.937±0.0230
		Media control absorbance	0.065±0.0016
2	MCF-7	Absorbance of cell control	0.575±0.0134
		Media control absorbance	0.053±0.0005
3	HeLa	Absorbance of cell control	0.709±0.0019
		Media control absorbance	0.082±0.0032
4	Vero	Absorbance of cell control	0.478±0.0161
		Media control absorbance	0.061±0.0033

To calculate the % cell viability, we need the cell control absorbance and media control absorbance of each cell line shown in Table 2. So in Table 3 shows the Cell Viability Value of ethanol extract of patikala leaves (*Etlingera elatior* (Jack) R.M. Smith) against cancer cells from North Luwu. From the data in the table shows the greater the concentration of ethanol extract against Widr, MCF-7, Hela cancer cells and normal cells (vero cells), the smaller the % cell viability.
Table 4 shows the activity of ethanol extract of patikala leaves from North Luwu against Widr, MCF-7, Hela and normal cells (vero cells).

Different concentrations of the ethanol extract (250 µg/mL, 125 µg/mL, 62.5 µg/mL, and 31.25 µg/mL) were tested against the WiDr cancer cells. The extract exerted significant inhibition of the WiDr cancer cells with an IC_{50} value of 67.5 µg/mL. However, against the MCF-7 cancer cells, the IC_{50} value of the extract was 213.5 µg/mL at concentrations of 1000 µg/mL, 500 µg/mL, 125 µg/mL, and 62.5 µg/mL. The MCF-7 cells were employed in this study because of their unique representation of human breast cancer. MCF-7 cell culture has several characteristics in different mammary epithelium including its ability to produce estradiol via cytoplasmic receptors and its ability to form a dome.[12]

Similarly, extract concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL and 125 µg/mL were employed against the HeLa cancer cell lines. Based on the IC_{50} value, the ethanol extract of Patikala was active against HeLa cells with an IC_{50} value of 263.4 µg/mL and therefore classified as weak cytotoxicity. In addition, extract testing was also carried out against Vero cells, specifically, at concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL. Based on the IC_{50} value the ethanol extract of Patilaka obtained from North Luwu of Indonesia, exhibited no cytotoxic activity against Vero cells at an IC_{50} value of 592 µg/mL. This result showed that the extract may not be toxic to normal human cells.
This study also determined the percentage of cell viability, a value that indicates the number of live cells in the presence of a test agent. Probit analysis can be used to determine cell viability to obtain the IC_{50} value. The analysis relates the percentage of cell viability and log concentration as shown in Figure 1. The data obtained in this study showed that the cytotoxic activity shown by ethanol extracts of Patikala used in this investigation possess lower IC_{50} values and better cytotoxic activity of the test sample.

Table 3: Cell Viability Values of ethanol extract of patikala leaf (*Etlingera elatior* (Jack) R.M.Smith) against cancer cells of North Luwu origin

No.	Cancer Cell	Concentration (µg/mL)	Cell viability (%) Mean±SD
1	WiDr	250	0.92±0.165
		125	11.53±2.027
		62.5	59.74±3.254
		31.25	82.33±4.336
2	MCF-7	1000	3.74±0.378
		500	5.27±0.372
		125	75.36±1.738
		62.5	92.99±1.422
3	HeLa	1000	4.63±0.313
		500	13.37±1.293
		250	61.17±2.298
		125	76.22±4.640
4	Vero	1000	33.90±0.942
		500	67.99±2.556
		250	76.63±5.177
		125	83.98±0.603

The results obtained in testing the four samples against Vero cells (normal cells) have IC_{50} values > 501 µg/mL (non-toxic) suggestive of not being toxic to normal cells. IC_{50} value > 501 µg/mL means that an extract does not have cytotoxic activity[13].

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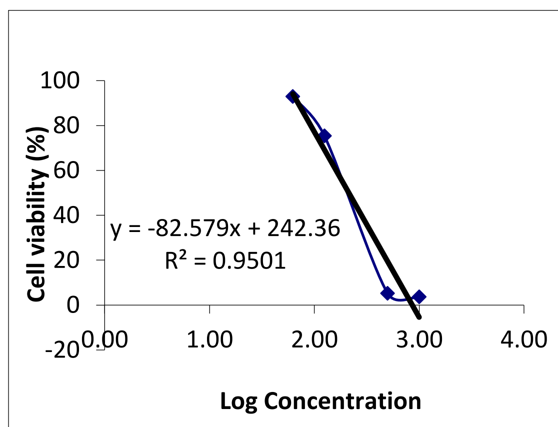
Table 4: Activity of ethanol extract of patikala leaves from North Luwu against several cell lines

Sample	Cancer cells	IC_{50} Value	Category
Ethanol extract of patikala leaves from North Luwu	WiDr	67.5 µg/mL	Medium
	MCF-7	213.5 µg/mL	Weak
	HeLa	263.4 µg/mL	Weak
	Vero	592 µg/mL	Non-toxic

This shows that patikala leaf extract does not have a cytotoxic effect on normal cells but is toxic only to WiDr Colon cancer cells in the moderate cytotoxic category. This also shows that patikala leaf extract has selective toxicity properties. MCF-7 breast cancer cells and cervical cancer (HeLa) with a weak cytotoxic category, in the IC_{50} value range of 200-500 µg/mL. These findings may be due to the unpurified nature of the extracts, and possibly, the activities may be more pronounced after further purification.

Furthermore, the anticancer activity of fractions from the ethanol extract of Patikala leaves. Fractions obtained from liquid-liquid partitioning of the crude extract of patikala leaves include n-hexane, ethyl acetate and water. Research shows that the fractionation process with organic solvents with different levels of polarity affects the type and level of extracted compounds.[14] n-hexane is a good solvent used for extracting non-polar compounds because it has the advantages of being volatile, stable, and selective.[15]



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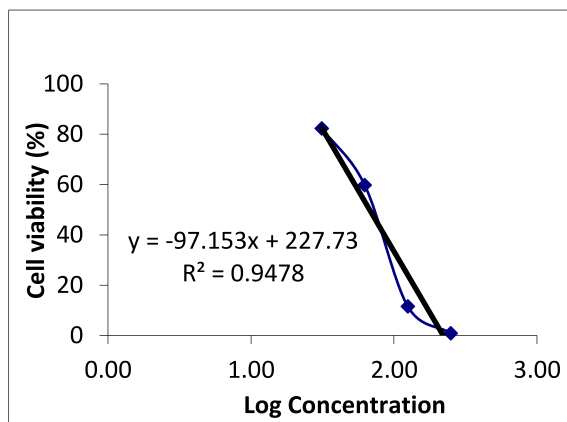

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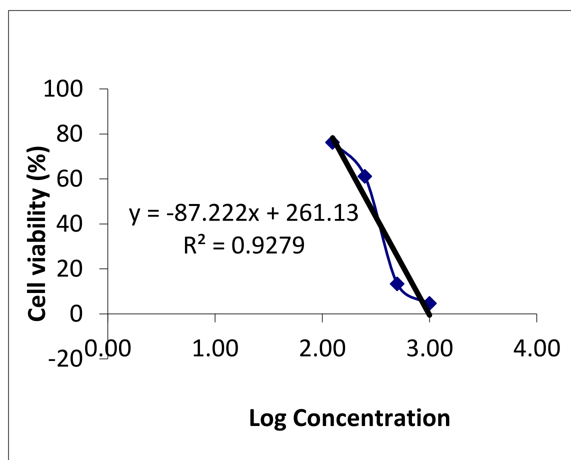

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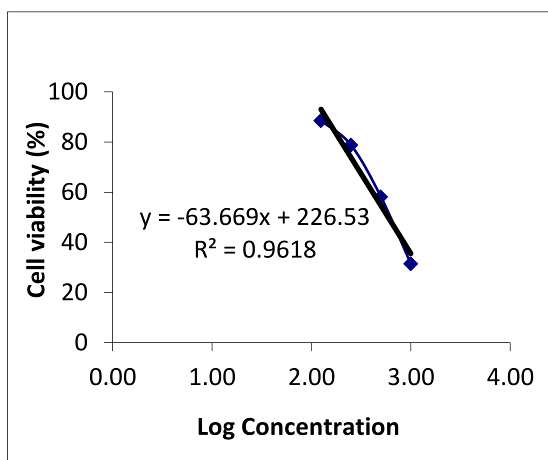

(D)

Figure 1: Linear equation to determine Cytotoxic activity of patikala leaf extract from North Luwu against cancer cells (A). Ethanol extract against WiDr cells, (B). Ethanol extract against MCF-7 cells, (C). Ethanol extract against HeLa cells, (D) Ethanol extract against Vero cells

Since ethyl acetate is a semi-polar solvent that attracts both polar and non-polar molecules, it was chosen as the solvent of choice to extract flavonoid chemical components from plants. Alkaloids, flavonoids, phenols, tannins, and saponins can all be extracted by semi-polar solvents.[16] The extractive yield of the patikala leaf fraction is presented in table 5. A high yield value shows the amount of bioactive components contained in the product. Yield is calculated as the ratio of the dry weight of the product generated to the weight of the material. A higher fraction yield indicates a higher concentration of compounds of interest in a raw material.[17] According to[18], the number of fractions obtained during the fractionation process is determined by the sample yield. Table 5 displays the extraction results, which indicates that the ethyl acetate fraction has the highest yield (39.77%) when compared to the n-hexane and water fractions.

To test the anticancer activity of the ethanol extract fraction of patikala leaves using the partition method, namely liquid-liquid extraction. Similar to extract testing, the absorbance of each fraction was first measured against Widr cells presented in table 6 and normal cells (vero cells) presented in table 10. To calculate % cell viability, we need cell control absorbance and media control absorbance of each cell line shown in table 7 for Widr cells and in table 11 for vero cells. So that the Cell Viability Value of fractions from ethanol extract of patikala leaves (*Etlingera elatior* (Jack) R.M. Smith) that have been partitioned against Widr cancer cells is presented in table 8 and for normal cells (vero cells) is presented in table 12. From the data in the table shows the greater the concentration of the sample against Widr cancer cells and normal cells (vero cells), the smaller the % cell viability.

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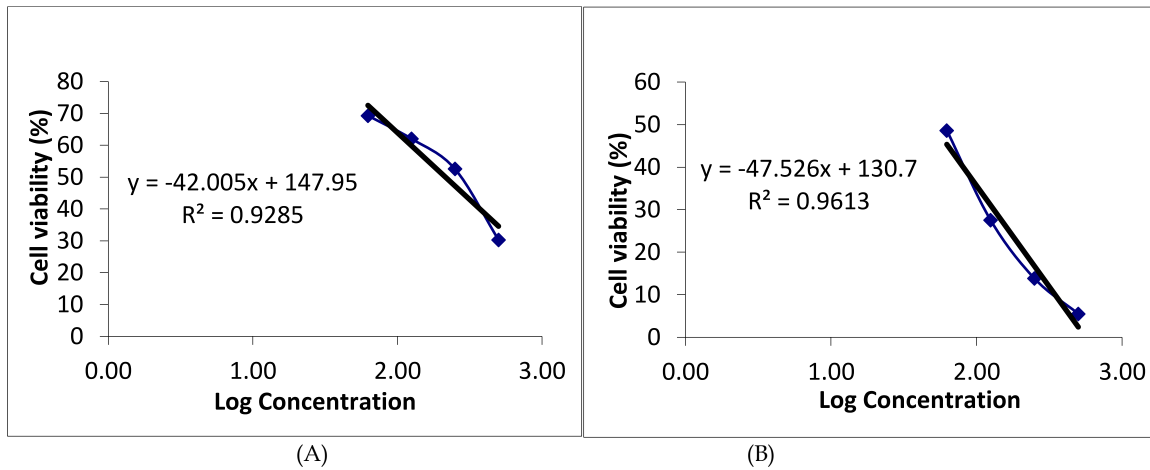
 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 5: Fraction yields of patikala leaf extract from North Luwu

Faction	Extract Weight (gram)	Faction Weight (gram)	Yield (%)
n-Hexan	10	3.0681	30.68%
Etil asetat	10	3.97659	39.77%
Water	10	2.95531	29.55%

Table 6: Cell Absorbance Results of Patikala Leaf Fraction (*Etlingera elatior* (Jack) R.M.Smith) against WiDr cancer cells from North Luwu

No.	Fraction	Concentration (µg/mL)	Absorbance Mean±SD
1	n-Hexan	500	0.226±0.0042
		250	0.357±0.0010
		125	0.413±0.0007
		62.5	0.456±0.0083
2	Ethyl acetate	500	0.080±0.0018
		250	0.129±0.0059
		125	0.210±0.0232
		62.5	0.334±0.0062
3	Water	500	0.291±0.0488
		250	0.420±0.0097
		125	0.494±0.0098
		62.5	0.525±0.0174



(A) (B)

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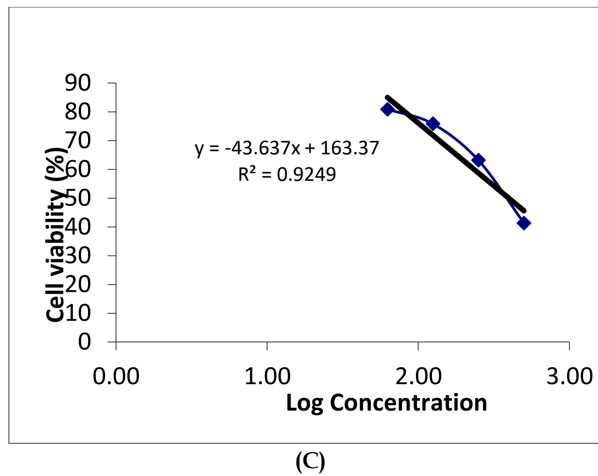

(C)

Figure 2: Liner equation to determine Cytotoxic activity of fractions from patikala leaf extract from North Luwu against WiDr cells (A). n-Hexan fraction against WiDr cells, (B) Ethyl acetate fraction against WiDr cells, and (C) Water fraction against WiDr cells.

Table 7: Cell Control and Media Control Absorbance Patikala Leaf Fractions (*Etlingera elatior* (Jack) R.M.Smith) from North Luwu against WiDr cancer cells

No	Fraction	Treatment	Mean±SD
1.	n-Hexan	Absorbance of cell control	0.637±0.0330
		Media control absorbance	0.047±0.0006
2	Ethyl acetate	Absorbance of cell control	0.637±0.0330
		Media control absorbance	0.047±0.0005
3	Water	Absorbance of cell control	0.637±0.0300
		Media control absorbance	0.047±0.0005

Table 8: Cell Viability Value of Patikala Leaf Fractions (*Etlingera elatior* (Jack) R.M.Smith) against WiDr cancer cells from North Luwu

No.	Fractions	Concentration (µg/mL)	Cell viability (%) Mean±SD
1	n-Hexan	500	30.31±0.7168
		250	52.54±0.1733
		125	62.03±0.1225
		62.5	69.30±1.4031
2	Ethyl acetate	500	5.53±0.3001
		250	13.84±0.9940
		125	27.53±3.9344
		62.5	48.65±1.0533
3	Water	500	41.34±8.2691
		250	63.16±1.6424
		125	75.81±1.6658
		62.5	80.91±2.9558

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ISSN 2616-0684 (Print)
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Table 9: The activity of fractions from ethanol extract of patikala leaves from North Luwu against WiDr cells

Faction	Cells	IC_{50} Value	Category
n-Hexan		214.7 µg/mL	Weak
Ethyl acetate	WiDr	49.9 µg/mL	Medium
Water		396.3 µg/mL	Weak

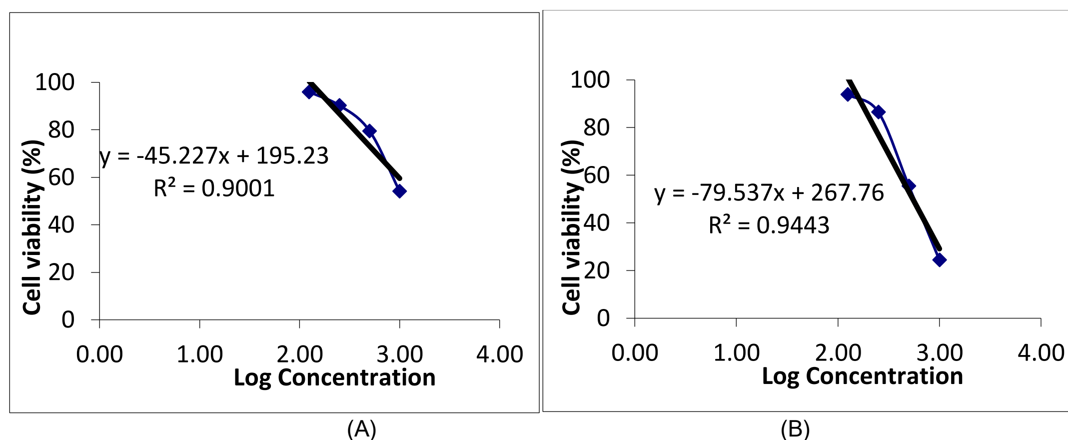
Table 10: Cell Absorbance Results of Patikala Leaf Fraction (*Etlingera elatior* (Jack) R.M.Smith) against Vero cells from North Luwu

No.	Fraction	Concentration (µg/mL)	Absorbance
			Mean±SD
		1000	0.384±0.0095
1	n-Hexan	500	0.545±0.0134
		250	0.612±0.0180

Table 11: Cell Control and Media Control Absorbance Patikala Leaf Fractions (*Etlingera elatior* (Jack) R.M.Smith) from North Luwu against Vero cells

No	Fraction	Treatment	Mean±SD
1.	n-Hexan	Absorbance of cell control	0.674±0.0141
		Media control absorbance	0.042±0.0003
2	Ethyl acetate	Absorbance of cell control	0.674±0.0141
		Media control absorbance	0.042±0.0003
3	Water	Absorbance of cell control	0.674±0.0141
		Media control absorbance	0.042±0.0003

Probit analysis can be used to determine cell viability to obtain IC50 values. This analysis relates the percentage of cell viability and log concentration to obtain a line equation to calculate the IC50 value. The line equation of patikala leaf fractions from liquid-liquid extraction, namely n-hexane fraction, ethyl acetate fraction and water fraction against Widr cells is shown in Figure 2 and against Vero cells in Figure 3.
Cytotoxic activity of patikala leaf fraction against WiDr cell culture with 4 concentration variations, specifically 62.5 µg/mL 125 µg/mL, 250 µg/mL, and 500 µg/mL is presented in Table 9. The results showed that the ethyl acetate fraction with an IC_{50} value of 49.9 µg/mL possesses the most potent cytotoxic activity from the highest concentration resulting in smaller cell viability compared to other samples. Whereas the n-hexane fraction showed a cytotoxicity effect with an IC_{50} value of 214.7 µg/mL and 396.3 µg/mL for the water fraction.
Based on Table 13, the cytotoxic activity of patikala leaf fractions from North Luwu against Vero cells shows that all samples were non-toxic to the Vero cells with an IC_{50} value > 501 µg/ml. The n-hexane fraction has an IC50 value of 1626.05 µg/mL, ethyl acetate fraction (546.8 µg/mL), and water fraction (7574.24 µg/mL).



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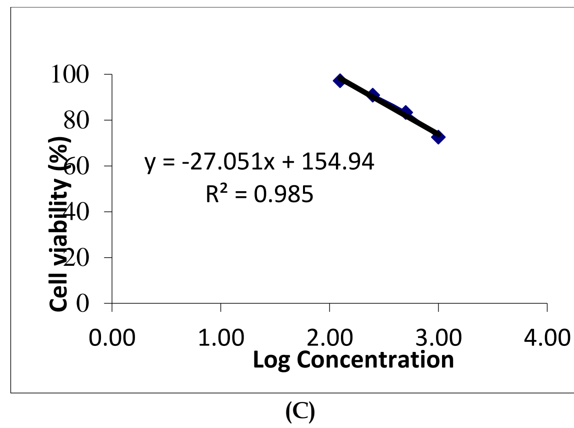
 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)



(C)

Figure 3: Liner equation to determine Cytotoxic activity of fractions from patikala leaf extract from North Luwu against Vero cells (A). Sample of n-Hexan fraction against Vero cells, (B) Ethyl acetate fraction against Vero cells, and (C) Water fraction against Vero cells.

Table 12: Cell Viability Value of Patikala Leaf Fractions (*Etlingera elatior* (Jack) R.M.Smith) against Vero cells from North Luwu

No.	Fraction	Concentration (µg/mL)	Cell viability (%) Mean±SD
1	n-Hexan	1000	54.11±1.5110
		500	79.57±2.1224
		250	90.28±2.8449
		125	95.93±1.6444
2	Ethyl acetate	1000	24.39±1.0291
		500	55.47±3.2888
		250	86.53±2.8446
		125	93.85±4.0954
3	Water	1000	72.59±2.1142
		500	83.34±2.1178
		250	90.87±1.6296
		125	97.22±1.7565

Based on previous research reports, the flavonoid and polyphenol contents of ethanol extracts of patikala leaves from North Luwu were 4.5994 mgEK/g.[6] Flavonoids are among the phytochemical substances with demonstrated anticancer activity. Quercetin molecules, which are classified as flavonoids, can dramatically reduce cell growth in WiDr cancer cells as well as induce apoptosis and cell cycle arrest. In previous research, it was reported that patikala plants contained flavonoid compounds that have strong anticancer activity and minimal effects on normal cells.[21] The distribution of secondary metabolites varies amongst samples, which influences the activity against cancer cells and accounts for the variation in IC_{50} values.[19]

Table 13: The activity of fractions of ethanol extract of patikala leaves from North Luwu against Vero cells

Faction	Cells	IC50 Value	Category
n-Hexan		1626.05 µg/mL	Non-toxic
Ethyl acetate	Vero	546.8 µg/mL	Non-toxic
Water		7574.24 µg/mL	Non-toxic

Conclusion

The study concluded that patikala leaf extract showed moderate to weak anticancer activity against several cell lines, with selective activity against WiDr cells but was not cytotoxic to Vero cell lines. The extract and fractions showed anticancer activities against all the cell lines used, but the ethyl acetate fraction was the most selective exhibiting a moderate cytotoxic. Meanwhile, all the fractions were not toxic to Vero cells.

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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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Acknowledgment

This research was funded by Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi, Republic of Indonesia (PDD Grant: 050/E5/PG.02.00.PL/2024).

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