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Cytotoxic activity of dichloromethane extract of soursop leaf (*Annona muricata* L.) against cancer

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ABSTRACT

Soursop leaves (*Annona muricata*) contain various secondary metabolite compounds with pharmacological benefits. This study foccused on the activity and chemical components cytotoxic active fraction of soursop leaf dichloromethane extract. From maceration of 535.8 g dried sample of *A. muricata* leaves we obtained extracts of *n*-hexane (18.4 g; 3.43 %). dichloromethane (11.4 g; 2.23 %). and methanol (48.7 g; 9.95 %). The dichloromethane extract was separated for its chemical components using gravity column chromatography resulting in 9 combined fractions. Toxicity screening test was done based on Bhrine Shrimp Lethality Test (BSLT) and cytotoxic test using MTT method 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide. The highest toxicity and cytotoxic test results were found in DMD 1 dichloromethane extract of *A. muricata* leaves with LC_{50} of 19.05 µg/mL and IC_{50} of 824.09 µg/mL. The results of DMD 1 gravity column re-chromatography produced 6-combined subfractions with the best separation pattern on DMD 1.4 and the results of GC-MS chromatogram analysis showed 29 peaks with the main component of fatty acid compounds namely Carbonic acid. but-2-yn-1-yl octadecyl ester (70.46 %). Potential anticancer compounds found were Phytol; 2-Hydroxy-1.1.10-trimethyl-6.9- epidioxydecalin and 2-Piperidinone. N-(4-bromo-n-butyl).

Keywords: *Annona muricata*. cancer. Cytotoxic activity. Dichloromethane extract. estracts. GCMS. Toxicity.

INTRODUCTION

Indonesia has rich natural resources. including herbal plants. which can be used as alternative medicine. Plants are an important source to discover new secondary metabolite bioactive compounds. Many compounds have been derived from plant species to treat several diseases. The pharmaceutical sector continues to encourage research and development foccused on new. innovative medicines. even tracing indigenous processes in using natural materials as traditional treatments in the medical system (24). One of the herbal plants known to have been widely used in Indonesia for traditional medicine is soursop (*Annona muricata*). Its leaves are used to treat diseases such as dermatitis. diarrhea. dysentery. fever. hypertension. diabetes. and cancer (4.13).

Cancer is a disease that caused 8.8 million deaths globally in 2015. It is characterized by abnormal cell proliferation due to malignant cell invasion (8). The abnormal reactions that occur lead to the development of tumors. immune system damage and disruption of normal body functions (20). Breast cancer is the second most common case among Asian women. affecting about 3 % individuals (17). Cancer treatment using chemotherapeutic agents has several limitations. such as resistance. side effects. and inadequate efficacy (18). Due to therapeutic inefficiency. there is a need to develop chemopreventive agents that are more effective and efficient. The use of chemopreventive agents can reduce the risk of

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cancer by inhibiting the initiation of cell mutation into malignant (preneoplastic lesions) by carcinogens. or by reversing cancer progression. One approach to finding chemopreventive compounds is through the exploration of natural materials. especially plants (7).

Preliminary tests are done to obtain anticancer compounds using the BSLT (Brine Shrimp Lethality Test) method. This toxicity test aims to determine the secondary metabolite compounds that can kill 50 % of test animal population (LC_{50}) (21). The cytotoxic activity against cancer cells *in-vitro* can be done with the MTT method (3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide). This method detects live cells. after exposure to extracts that have anti-cancer potential. Therefore. this method can be used to measure cytotoxicity. proliferation. or cell activation. The principle of this method measures the reduction of yellow tetrazolium salt to purple formazan crystals. Toxicity and cytotoxicity tests have a strong relationship. The strong toxicity can be a reference for making anticancer drugs. The fact that a substance has a strong LC_{50} doesn't necessarily mean it will be toxic to normal cells in the same way. The reason lies in the specificity of the substance's action. Many toxic substances act on specific targets or pathways within the body. leading to their toxic effects. Normal cells may not express the target or be as sensitive to the substance's effects. thereby reducing their toxicity compared to the target cells or organisms. Normal cells often have robust mechanisms for repairing damage or eliminating toxic substances. which can mitigate the effects of exposure. These mechanisms may include DNA repair pathways. antioxidant systems and detoxification enzymes. In contrast. cells that are the target of the toxic substance may lack such defences. making them more susceptible to damage and death. Additionally. the concentration required to achieve an LC_{50} in a toxicology study may not be relevant to exposures encountered in normal conditions. The concentration needed to kill 50 % of a population in a controlled laboratory setting may be much higher than that would be encountered in real-world scenarios.

This study aimed to isolate the chemical compounds contained in the dichloromethane extract of leaves of *A. muricata* and determine their cytotoxic activity using the BSLT and MTT assay methods and analyze the data by Gas Chromatography-Mass Spectrometry chromatogram to determine the cytotoxic of active compounds found in the active fraction. Some active compounds reported in previous researchers were : Annomuricin A. Annomuricin B and Muricatosin C. Annomuricin A compounds show ED_{50} values > 1.0 μg/mL against MCF-7 cells. ED_{50} > 1.0 μg/mL against HT-29 cells. $ED_{50} = 3.30 \times 10^{-1}$ μg/mL against A549 cells. and toxicity of $LC_{50} = 6.25 \times 10^{-1}$ μg/mL. Annomuricin B compound showed an ED_{50} value >1.0 μg/mL against MCF-7 cells. $ED_{50} = 4.35 \times 10^{-1}$ μg/mL against HT-29 cells. $ED_{50} = 1.59 \times 10^{-1}$ μg/mL against A549 cells. and toxicity of $LC_{50} = 6.87 \times 10^{-1}$ μg/mL (26). Muricatocin C compound showed $ED_{50} = 6.45 \times 10^{-2}$ μg/mL against MCF-7 cells. $ED_{50} = 9.09 \times 10^{-2}$ μg /mL against A549 cells. $ED_{50} = 1.48$ μg/mL against HT cells -29. and toxicity of $LC_{50} = 6.04 \times 10^{-1}$ μg/mL (27).

MATERIALS AND METHODS

The materials used were : solvent *n*-hexane (99 % purity Merck. Germany). dichloromethane (99 % purity Merck. Germany). methanol (70 % purity Merck. Germany). silica gel G60 (0.2–0.5 mm. Germany). seawater. dimethyl sulfoxide. deionized water. sodium bicarbonate. dimethyl sulfoxide. trypan blue. fetal bovine serum. penicillin-streptomycin. carbon dioxide. trypsin. phosphate buffered saline. MTT 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium-bromide and DMEM (Dulbecco's Modified Eagle Medium).

The instruments used were an ELISA reader (Enzyme-Linked Immunosorbent Assay) Thermo Fisher Scientific microplate spectrophotometer with a wavelength of 595 nm for anticancer tests. and GC-MS (Gas Chromatography–Mass Spectrometry) Thermo Scientific^{TM} for chemical compound analysis.

METHODS

(I). Sample Preparation

A. muricata leaves were obtained from Montasik. Aceh Besar. Aceh-Indonesia with latitude 5.516658° and longitude 95.47696°. The samples were cleaned and dried in shade at room temperature for 2 weeks. The dried samples were ground into powder weighing 535.8 g. The powder was macerated using an *n*-hexane solution. The macerated dregs were dried and 10 % ammonia was added. Maceration was continued with dichloromethane solvent and then methanol. Filtrates of the three solvents were dried using a rotary evaporator under vacuum conditions and stored in a refrigerator for further characterization preparation (6).

(II). Phytochemical Analysis

A. muricata leaf extract was subjected to phytochemical screening to determine the content of secondary metabolite compounds by qualitative detection including alkaloid. steroid. terpenoid. saponin. phenolic. and flavonoid tests using the method of Harborne (10) with slight modifications.

(III). Cytotoxicity

(i). BSLT Assay

10 *Artemia salina* larvae aged 48 h were exposed to 5 mL of test solution concentration (50. 100. 250. 500. and 1.000 µg/mL) after dilution with 5 % dimethyl sulfoxide in triplicate. The negative control used was seawater. The test was carried out under 15-watt lamp lighting and observed for 24 h (14). The LC_{50} (Median Lethal Concentration) value was obtained from the regression equation. the relationship between the probit value from % dead animals corrected (Y) and the log concentration (X). The % dead animals corrected value uses the following equation (1):

$$\text{\% Dead animals corrected} = \frac{(\text{\% Dead animal samples} - \text{\% Negative control dead animals})}{(100 - \text{\% Negative control dead animals})} \quad (1)$$

(ii). MTT Assay

MCF-7 cells were grown in DMEM (Dulbecco's Modified Eagle Medium). The medium was filtered and 5 % FBS (Fetal Bovine Serum) and 1 % Pen-Strep (Penicillin-Streptomycin) antibiotic were added. Confluent cells were subcultured and 5 mL of 0.125 % trypsin was added to the culture flask. Incubation was carried at 37 °C with 5 % CO_2 humidity for 5 min so that the cells were released from the culture flask. A total of 5.000 cells/100 µL of media (per well) were grown in 96 well plates. Cell counting was done using a Neubauer Haemocytometer. *A. muricata* leaf extract with 250. 125. 62.5 and 31.25 µg/mL concentration was added at 100 µL/well. Tests were done in triplicate with cells without sample treatment as control cells (15). Cell morphology images were taken after the samples were treated. Next. read the OD (Optical Density) value using a microplate ELISA reader at a wavelength of 595 nm after adding MTT 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium-bromide. Calculation of the IC_{50} (Half Maximal Inhibitory Concentration) value is obtained from the relationship between the percent inhibition (Y) and the concentration of the sample solution (X) in the linear regression equation. Inhibition (%) value was obtained using the following equation (2):

$$\text{Inhibition (\%)} = 1 - \left(\frac{\text{Extract absorbance}}{\text{Control absorbance}}\right) \times 100\ \% \qquad (2)$$

(IV). Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical compounds contained in the extract were identified using GC-MS. The carrier gas used was Helium (Ultra High Purity 99.99 %) at a flow rate of 0.8 mL/minute with 1.000 µL of sample injected. GC settings were as under: PrepRun Timeout; 999 min. Equilibration Time; 0.50 min FrontInlet (FlowMode; FlowCtrl. SplitFlow.Nominal; 50.0 mL/min. GasSaverFlow; 10 mL/min. GasSaverTime; 1 min. Temperature. Nominal; 250 °C). FrontColumn (Description; TG-5MS. Length; 30 m. NominalID; 0.250 mm. Film Thickness; 0.25 µm). The type of analysis used is Single with Injection Mode; Basic. and Rapid Mode; Disable. The type of syringe (Volume 10.000 µL and Needle Length 57.000 mm). Compounds found in the extract were seen for their peak-peak similarity to the NIST08 (National Institute of Standards and Technology) database.

RESULTS AND DISCUSSION

1.1. Samples and Extraction Yields

Extraction of *A. muricata* leaf used three solvents with graded polarity. namely *n*-hexane. dichloromethane. and methanol. The three types of solvents used have different levels of polarity. ranging from polar to semi-polar to non-polar. This aimed to ensure that the chemical components of the sample can be separated properly based on their polarity level. The results of *A. muricata* leaf extraction can be seen in Table 1. Methanol extract of *A. muricata* leaves has the highest yield value (9.95 %) compared to *n*-hexane (3.43 %) and dichloromethane (2.23 %) extracts. This showed that there are more bioactive components contained in the methanol extract because methanol is a universal solvent that can attract polar to non-polar compounds so that the extracted compounds consisted not only of polar compounds but also other compounds. This result is relevant to research by Dawra *et al.* (6) on *Salvia fruticosa* plants. where the highest yield (%) was obtained in the methanol extract (9.2 %). followed by the n-hexane extract (3.6 %). and dichloromethane extract (3.4 %). The methanol extract gave the highest yield percentage.

Table 1. Extraction yields from *A. muricata* leaf

Extract	Sample weight (g)	Extract weight (g)	Yield (% b/b)	Color
DMH	535.8	18.4	3.43	Blackish-green
DMD	510.4	11.4	2.23	Blackish-brown
DMM	489.0	48.7	9.95	Blackish-green

DMH: n-hexane; DCM: dichloromethane; DMM : methanol extract from *A. muricata* leaf

1.2. Phytochemical Analysis

Phytochemical test results obtained from the three extracts showed that the *n*-hexane extract contained steroid and terpenoid compounds. Dichloromethane and methanol extracts contained phenolic compounds. flavonoids. steroids. terpenoids. saponins and alkaloids with different reagents. The dichloromethane extract was positive for alkaloids in the Dragendorf reagent and the methanol extract was positive for alkaloids in the Dragendorf and Wagner reagents (Table 2). These results were in line with the phytochemical results reported by several other researchers that *A. muricata* leaves contains phenolic compounds (16). terpenoids and steroids. alkaloids (4). flavonoids. tannins. triterpenoids. and carbohydrates (28). The results of phytochemical screening aimed to provide initial data regarding the chemical components contained in the extract before toxicity and cytotoxic tests were done.

Table 2. The phytochemical screening tests from *A. muricata* leaf

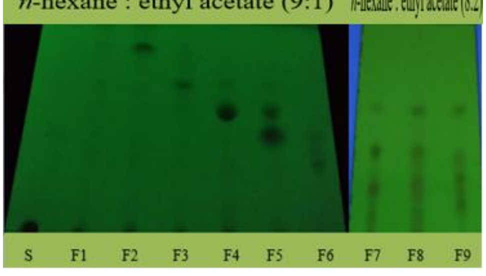
No	Phytochemical test	Reagent test	Extract sample		
			DMH	DMD	DMM
1	Alkaloids	Dragendorf	-	+	+
		Mayer	-	-	-
		Wagner	-	-	+
2	Phenolic	$FeCl_3$	-	+	+
3	Flavonoids	Mg + HCl	-	+	+
4	Steroids	Liebermann-Burchard	+	+	+
5	Terpenoids	Liebermann-Burchard	+	+	+
6	Saponins	Water and shaken	-	+	+

DMH: n-hexane; DCM: dichloromethane; DMM : methanol extract from *A. muricata* leaf

1.3. TLC Analysis

The KLT results of the fractions obtained from the separation of chemical compounds using gravity column chromatography showed a total of 262 fractions. Each fraction was monitored based on similar stain markers for each fraction. The fractions having similar stain patterns were combined into 9 combined fractions namely DMD 1 (1-15). DMD 2 (16-30). DMD 3 (31-100). DMD 4 (101-149). DMD 5 (150-169). DMD 6 (170-185). DMD 7 (186-250). DMD 8 (251-255). and DMD 9 (256-262) (Figure 1).

a)



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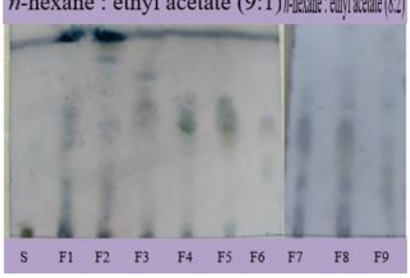


Figure 1. TLC of the combined fractions DMD 1 to DMD 9 (a) Elution results under a 254 nm UV lamp. (b) Elution results with Vanillin Sulphate reagent.

The combined fraction DMD 1 has strong toxicity activity (Table 3). so gravity column re-chromatography was performed. The obtained KLT results show a total of 101 subfractions with combined subfractions based on the similarity of stain patterns. namely DMD 1.1 (1-12). DMD 1.2 (13-19). DMD 1.3 (20-39). DMD 1.4 (40-49). DMD 1.5 (50-66). and DMD 1.6 (67-101) were shown (Figure 2). DMD 1.4 is the result of KLT with the best stain separation pattern. so it is analyzed using Gas Chromatography-Mass Spectrometry.

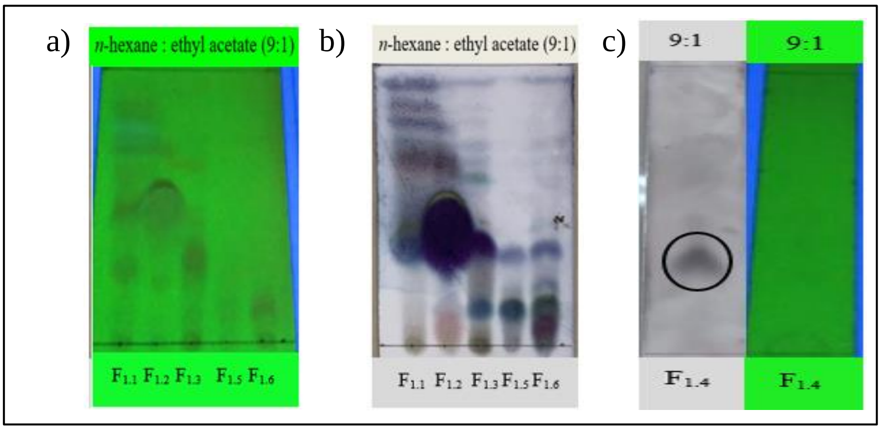


Figure 2. TLC of the combined subfractions DMD 1.1 to DMD 1.6. (a) Elution results under a 254 nm UV lamp. (b) Elution results with Vanillin Sulfate reagent. (c) Combined subfraction of DMD 1.4 is the best separation.

1.4. BSLT Test from the *A. muricata* extract and faction groups

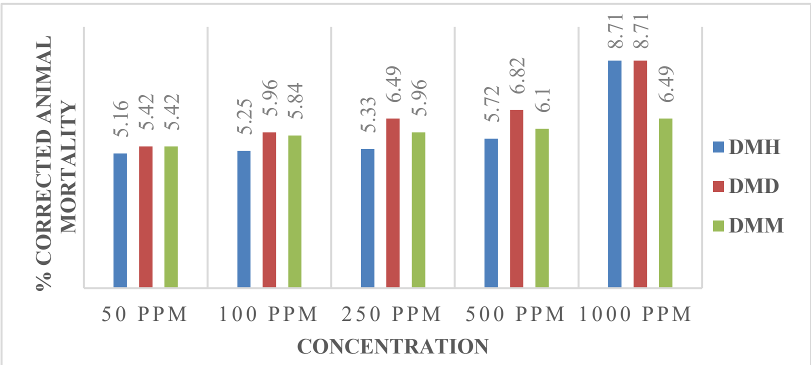


Figure 3. The relationship between the concentration of the *n*-hexane. dichloromethane. and methanol extract test solutions of *A. muricata* leaves on the percentage of corrected larval death

Testing with the BSLT method was introduced by Meyer *et al*. (14) using bioindicators in the form of shrimp larvae (*A. salina*). This method is considered sensitive to various substances. so it is useful as a simple and quick bioassay to predict the toxicity of a plant extract based on the LC_{50} value (3.9). as well as being the first step to discover of cytotoxic and antitumor compounds. An LC_{50} value <100 µg/mL indicates an association with cytotoxic and antitumor activity (9). according to what was obtained from research results. The LC_{50} value of n-hexane. dichloromethane and methanol extract of *A. muricata* leaves (Figure 3) has very strong toxicity activity. namely DMM (LC_{50} 14.79 µg/mL) followed by DMD (LC_{50} 22.38 µg/mL) and DMH (LC_{50} 29.51 µg/mL). The combined fraction from the isolation of dichloromethane extract from *A. muricata* leaves was also tested for toxicity (Table 3). The correlation coefficient (R^2) value was close to 1. indicating that the factor influencing the death of the test larvae is the result of the compounds contained in the extract. but if away from number 1 indicates that there are other factors (outside the compounds contained in the extract) that cause the death of the test larvae.

Table 3. LC_{50} (µg/mL) of the combined fraction from soursop leaf dichloromethane extract

Extract	Percent mortality at 24 hours (%)					LC_{50} (µg/mL)
	50 µg/mL	100 µg/mL	250 µg/mL	500 µg/mL	1.000 µg/mL	
DMD 1	56.67	60.00	67.67	70	93.33	19.05
DMD 2	13.33	33.33	43.33	56.67	63.33	354.81
DMD 3	36.67	43.33	50.00	53.33	56.67	331.13
DMD 4	10.00	23.33	33.33	46.67	53.33	741.31
DMD 5	23.33	36.67	46.67	53.33	60.00	363.07
DMD 6	26.67	40.00	46.67	56.67	63.33	323.59
DMD 7	30.00	43.33	56.67	83.33	96.67	109.64
DMD 8	60.00	66.67	80.00	90.00	96.67	53.70
DMD 9	63.33	76.67	83.33	96.67	100.00	30.90

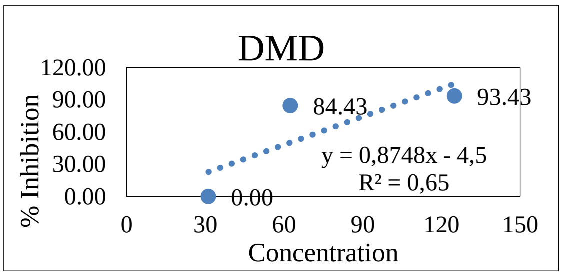
DCM: dichloromethane extract from *A. muricata* leaf

Calculation of LC_{50} values of the 9-groups of fractions of dichloromethane extract of *A. muricata* from the highly toxic DMD 1 (LC_{50} 19.05 µg/mL). toxic DMD 2 (LC_{50} 354.81 µg/mL). DMD 3 (LC_{50} 331.13 µg/mL). DMD 4 (LC_{50} 741. 31 µg/mL). DMD 5 (LC_{50} 363.07 µg/mL). DMD 6 (LC_{50} 323.59 µg/mL). DMD 7 (LC_{50} 109.64 µg/mL). DMD 8 (LC_{50} 53.70 µg/mL) and DMD 9 (LC_{50} 30.90 µg/mL). Based on the highly toxic LC_{50} value. DMD 1 was tested for cytotoxicity using the MTT assay method. This was done to find out whether the chemical components contained in the DMD 1 extract could function as anticancer agents. Based on the LC_{50} value which is classified as toxic to test animals. *Artemia salina* larvae will be followed by a cytotoxic test using the MTT assay method to detect the presence of antineoplastic activity.

1.5. MTT Assay from the *A. muricata* extract and faction groups

The extracts with IC_{50} <30 µg/mL have promising cytotoxic activity (22). Cytotoxic testing was conducted on dichloromethane extract and dichloromethane extract of combined fraction 1 of *A. muricata* leaves. as an advanced test of the extract that has the strongest toxicity level (Figure 3 and Table 3). The test results showed that the dichloromethane extract was strongly cytotoxic (DMD IC_{50} 62.29 µg/mL) and the combined fraction 1 dichloromethane extract was not cytotoxic (DMD 1 IC_{50} 824.09 µg/mL). Measurements were made to see the ability of the extracts to inhibit cancer cell growth based on the percentage of inhibition (Figure 4) which is based on the OD (Optical Density) value as a reading of the absorbance of formazan resulting from the reduction reaction of succinate dehydrogenase enzyme in living cells with tetrazolium salt. using an ELISA reader at λ = 595 nm. Cell cycle profile observations were also made on both extracts as shown in Figures 5 and 6.

a)



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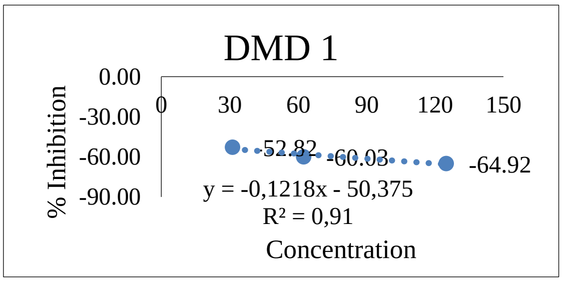

Figure 4. Graph of the relationship between extract concentration and % inhibition (a) Dichloromethane extract of *A. muricata* leaf. (b) Dichloromethane extract of fraction groups 1 (DMD 1) from *A. muricata* leaf.

Cell cycle profiling was done by flow cytometer. the extract treatment increased the cell population in the sub G1 phase undergoing apoptotic events. without going through cell cycle inhibition. Apoptosis or programmed cell death is a cellular process that occurs in normal and pathological physiological conditions (25). Overall. *A. muricata* leaf extract showed cytotoxic properties to inhibit the proliferation of MCF-7 cancer cells *in-vitro* at higher concentrations than the cell control. The morphological appearance of MCF-7 cancer cells in the treatment of 250 µg/mL concentration of *A. muricata* leaf dichloromethane extract showed some black stains as dead cells (cell apoptosis) can be seen (Figure 5). while the combined fraction 1 dichloromethane extract is not very visible (Figure 6). this may be due to the content of compounds contained in the combined fraction is dominated by other compounds that are less active anti-cancer. The ability of *A. muricata* extract to reduce the rate of cancer cell proliferation was also confirmed by several researchers. including dried fruits (H_2O: Cet 50 %) has IC_{50} > 200 µg/mL against cell lines MCF-10A. MCF-7. MDA-

MB-231. and BC MDA-MB-468 with IC_{50} = 4.8 µg/mL (5). Leaf/stem (DMSO) against PC FG/COLO357 and PC CD18/HPAF cell lines had IC_{50} = 200 µg/mL and 73 µg/mL (Torres et al.. 2012). leaf (*n*-Hex. EtOAc. MeOH) against HT-29 cell line had IC_{50} of 14.93 µg/mL. 4.29 µg/mL. IC_{50} > 100 µg/mL and against HCT-116 cell line had IC_{50} of 12.26 µg/mL. 3.91 µg/mL. IC_{50} > 100 µg/mL (16).

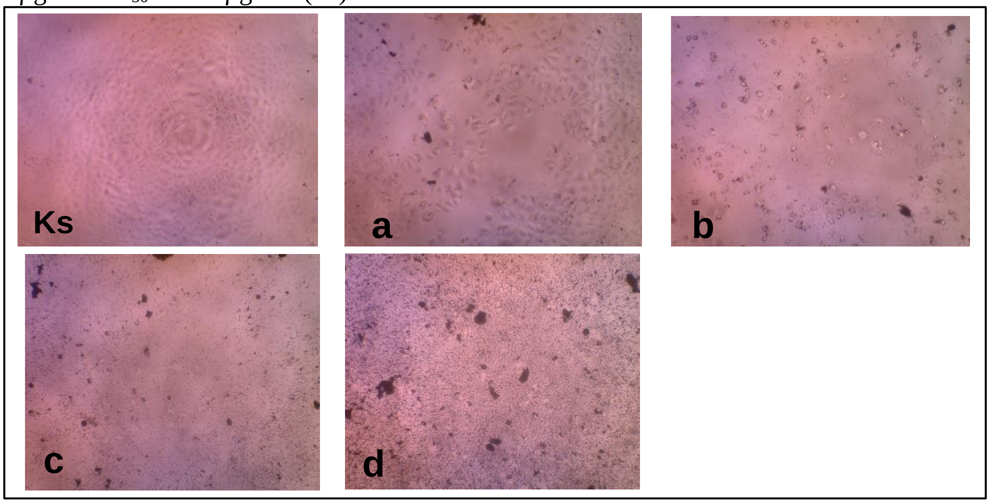

Figure 5. Morphology of MCF-7 Cells in the treatment of dichloromethane extract of *A. muricata* leaf (DMD). concentrations 31.25. 62.5. 125. and 250 µg/mL (a–d) compared to Control cells (Ks).

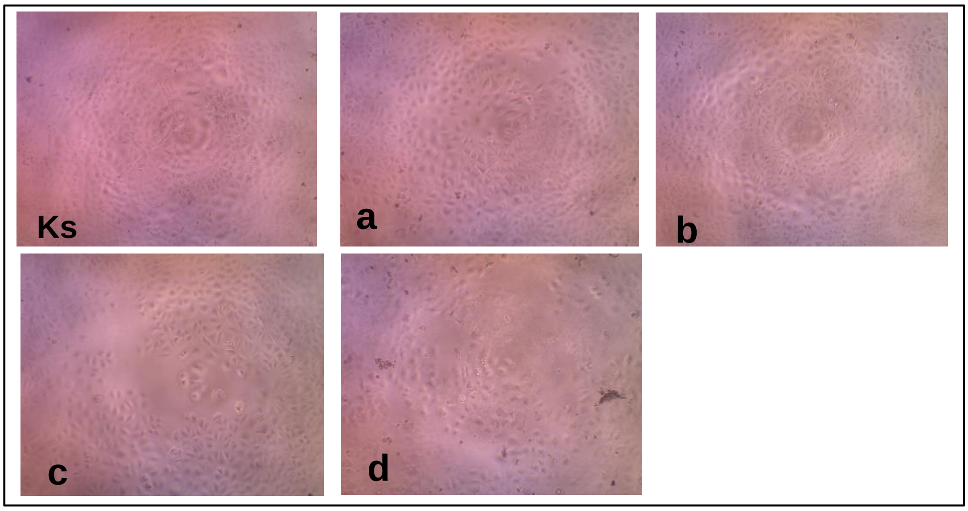

Figure 6. Morphology of MCF-7 Cells in the treatment of dichloromethane extract of fraction groups 1 from *A. muricata* leaf (DMD 1). concentrations 31.25. 62.5. 125. and 250 µg/mL (a–d) compared to Control cells (Ks).

1.6. Phytochemical profile using GC-MS of the DMD 1.4 from *A. muricata* dichloromethane extract

The chromatogram of GC-MS analysis was done on DMD 1.4 dichloromethane extract of *A. muricata* leaves (Figure 7). The names of the compounds along with their retention times and peak areas (%) are presented in Table 4. There is one compound with

Table 4. Compounds identified using GC-MS in DMD 1.4 from *A. muricata* dichloromethane extract

No	Compounds	Retention Time (min)	Relative Area (%)	Similarity (%)
1	Carbonic acid. but-2-yn-1-yl octadecyl ester	37.07	70.46	72.8
2	Hexane. 1-(3-butenyloxy)	7.75	6.11	81.9
3	Ethanone. 1-(3-ethyloxiranyl)	7.48	5.03	82.5
4	7.9-Di-tert-butyl-1-oxaspiro(4.5)deca-6.9-diene-2.8-dione	31.74	2.02	90.2
5	Phytol	35.34	1.92	91.1
6	2-Hydroxy-1.1.10-trimethyl-6.9-epidioxydecalin	35.15	1.44	74.0
7	1-Dodecanamine. N.N-dimethyl	22.56	1.21	85.6
8	11-Methyldodecanol	36.71	1.06	72.8
9	1-Dodecanamine. N.N-dimethyl	19.69	0.88	83.4
10	Cholesterol	50.47	0.88	83.1
11	2-Piperidinone. N-(4-bromo-n-butyl)	34.82	0.80	77.2
12	Phthalic acid. di(2-propylpentyl) ester	42.55	0.78	86.2
13	2-Pentadecanone. 6.10.14-trimethyl	30.19	0.75	85.9
14	Heptadecanoic acid. heptadecyl ester	36.61	0.73	73.4
15	cis-Z-a-Bisabolene epoxide	28.33	0.67	80.2
16	2-Piperidinone. N-(4-bromo-n-butyl)	37.92	0.66	74.5
17	3.7.11.15-Tetramethylhexadecane-1.2.3-triol triacetate	38.67	0.56	78.1
18	Hexadecanal. 2-methyl	31.60	0.52	77.0
19	2-Pentene. 4.4-dimethyl	8.04	0.47	77.2
20	2-Piperidinone. N-(4-bromo-n-butyl)	39.51	0.43	77.4
21	6-Octadecenoic acid. methyl ester. (Z)	34.19	0.42	83.7
22	Hexadecanoic acid. Methyl ester	33.09	0.42	71.6
23	4-Methyldocosane	36.20	0.41	73.0
24	Z-8-Methyl-9-tetradecenoic acid	38.52	0.35	79.6
25	4-Propionyloxytridecane	40.19	0.26	69.7
26	Acetone.1-(4-(dimethylaminoethoxy) phenyl)	27.18	0.24	69.3
27	N-Dimethylaminomethyl-tert.-butylisopropylphosphine	25.29	0.23	77.0
28	2.2-Dimethyl-6-methylene-1-(3.5-dihydroxy-1-pentenyl)cyclohexan-1-perhydrol	53.01	0.22	56.3
29	Heptadecanoic acid. 10-methyl-. methyl ester	34.70	0.07	74.3

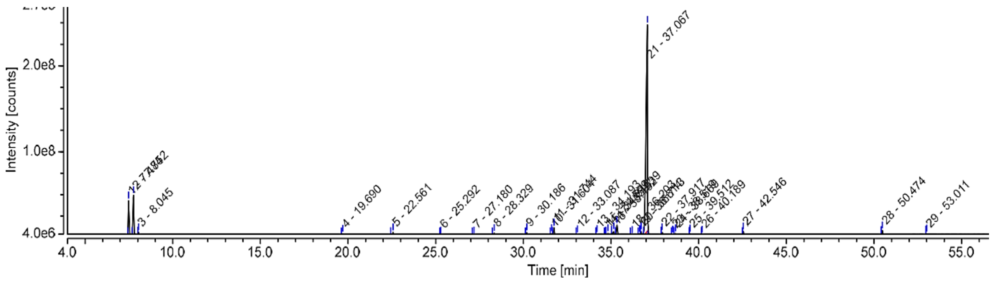

Figure 7. GC-MS chromatogram of DMD extract from *A. muricata* leaf.

the largest peak area. namely Carbonic acid. but-2-yn-1-yl octadecyl ester (70.46 %). Other compounds with a peak area > 1 % include Hexane. 1-(3-butenyloxy) (6.11 %). Ethanone. 1-(3-ethyloxiranyl) (5.03 %). 7.9-Di-tert-butyl-1-oxaspiro(4.5)deca-6.9-diene-2.8-dione (2.02 %). Phytol (1.92 %) has the potential as a cytotoxic agents from the diterpene group that is good for cancer therapy through the fragmentation mechanism (1). 2-Hydroxy-1.1.10-trimethyl-6.9- epidioxydecalin (1.44 %) as an anticancer by affecting high cell apoptosis stimulation (20). 1-Dodecanamine. N. N-dimethyl (1.21 %). and 11-Methyldodecanol (1.06 %). The compound 2-Piperidinone. N-(4-bromo-n-butyl) can inhibit cancer cell proliferation. Piperine and piperidine compounds are alkaloid amide compounds that work by activating cancer apoptosis so that cell division is controlled (2.12). This research still has several shortcomings. such as further re-chromatography of the DMD 1.4 extract to obtain pure isolates and carrying out toxicity and cytotoxic tests on pure isolates. then characterization was done using NMR to find out more about compounds that play a role in anticancer activity and can be used as anticancer drugs.

CONCLUSIONS

Dichloromethane extract of *A. muricata* leaves has relatively strong toxicity with an LC_{50} value of 50 22.38 µg/mL so it can be an anticancer agent. Separation of the chemical components of the dichloromethane extract of *A. muricata* leaves resulted in 9 combined fractions. DMD 1 had the highest toxicity value with an LC_{50} of 19.05 µg/mL than other fractions combined. Therefore. a cytotoxic test was done to determine the good IC_{50} value as an anticancer. The IC_{50} value of DMD 1 extract from the cytotoxic test was 824.09 µg/mL and gravity column rechromatography was done to produce 6 combined subfractions. GC-MS chromatogram analysis of DMD 1.4 extract produced 29 peaks with compounds that have potential anticancer properties. including Phytol; 2-Hydroxy-1.1.10-trimethyl-6.9-epidioxidecaline; and 2-Piperidinone. N-(4-bromo-n-butyl).

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CONFLICT OF INTEREST

The authors announce that they have no conflicts of interest.

DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We have not excluded any author who substantially contributed to this manuscript. We have followed the ethical norms established by our respective institutions.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct.

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