

# Cytotoxicity Activity, Metabolite Profiling, and Isolation Compound from Crude Hexane Extract of *Cleome rutidospermae*

Budiman Yasir<sup>1,2,3</sup>, Abdul Rahim<sup>2</sup>, Subehan Lallo<sup>2</sup>, Yohei Saito<sup>4</sup>, Kyoko Nakagawa-Goto<sup>4</sup>, Abdul Rohman<sup>5</sup>, Gemini Alam<sup>2\*</sup>

## Abstract

**Objective:** This study isolated the chemical compounds and evaluated the cytotoxic activity of the crude hexane extract of *Cleome rutidospermae* herb (CRH). **Methods:** The isolate was purified using silica gel, column chromatography, and preparative thin layer chromatography (PTLC). Furthermore, the structure of the compounds was identified by spectroscopic methods using 1D, 2D NMR, and mass spectrometry. The cytotoxic activity of CRH at a concentration of 20 ug/mL was also tested against MCF-7, A549, KB, KB-VIN, and MDA-MB-231 cancer cells using the sulforhodamine B (SRB) method. **Results:** The CRH contained compounds of unsaturated fatty acid, saturated fatty acid, lipid, glycerol,  $\omega$ -3 fatty acid, and cholesterol. Two compounds were obtained from the plant, and their structures were identified as (1) Stigmasta-5,22-dien-3-ol (STML) and (2) 1,2-Benzene dicarboxylic acid, 1,2-bis (2-Ethylhexyl) esters (DEHP). These compounds were reported in this plant for the first time. In comparison, CRH had % growth inhibition in the proliferation of MCF-7 cells up to 28.1%, with cancer cells A549, KB, KB-VIN, and MDA-MB-231 by >50% Compared to the negative DMSO of 0.20%, while the positive control could inhibit the growth of all cancer cells (100%). **Conclusion:** Our findings suggested that crude herb from the plant CRH was the potential for breast cancer treatment.

**Keywords:** *Cleome rutidospermae*- Stigmasta-5,22-dien-3-ol- Bis(2-Ethylhexyl) esters (DEHP)- Cytotoxic

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## Introduction

Medicinal plants have been proven to be an alternative approach for treating various health conditions. Although synthetic drugs have taken traditional healing to some degree, the revival and attention of herbal medicines are very important (Mirzaeian et al., 2021). Medicinal plants are affordable and eco-friendly, with fewer side effects than synthetic drugs. In fact, among the modern medicines currently in use, approximately 40% come from nature, while 60% of anticancer and 75% of infectious diseases drugs are natural or derivatives. Furthermore, some of these plants have attracted scientists' interest in investigating cancer treatment. Additionally, phytoconstituents have been essential in developing clinically valuable prospects for treating neoplasms (Gupta et al., 2021).

*Cleome rutidospermae* is an annual herbaceous plant known as the Fringed Spider Flower. Besides being a weed, it is also an important medicinal plant widely found in the tropics. Furthermore, it is ayurvedically, Greek,

and local Kabiraj used to manufacture bread. Its roots or seeds are used as a stimulant, antiscorbutic, anthelmintic, rubefacient, vesicant, and carminative. The plant is also an analgesic, antipyretic, anti-inflammatory, antimicrobial, antioxidant, anti-seizure, anti-diabetic, and diuretic, with wound healing and laxative activity (Ghosh et al., 2019). Its herb contains water (81.0 g), energy (239 kJ/57 kcal), protein (5.5 g), fat (0.9 g), carbohydrate (10.1 g), Fiber (1.7 g), Ca (454 mg), Mg (38 mg), P (59 mg), and Fe (2.7 mg) per 100 g of edible portion, tannins, lipids, amino acids, flavonoids, cardiac glycosides, alkaloids, steroids, saponins, terpenoids, polyphenols, and phlorotannin pentose and reducing sugars (Ghosh et al., 2019).

Natural ingredients are considered an essential source of discovery for anticancer treatments, and many cytotoxic drugs in clinics and hospitals today are derived from plants and other natural sources (Sammar et al., 2019). Phytochemicals from plant extracts are included in important sources of natural products that have shown excellent cytotoxic activity. However, plants of

<sup>1</sup>*Sekolah Tinggi Ilmu Farmasi Makassar, Makassar, 90242, Indonesia.* <sup>2</sup>*Department of Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia.* <sup>3</sup>*Faculty of Health Sciences, Almarisah Madani University, Makassar, 90245, Indonesia.* <sup>4</sup>*School of Pharmaceutical Sciences, College of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, 920-1192, Japan.* <sup>5</sup>*Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, 55281, Indonesia.* \*For Correspondence: daengta007@yahoo.com

different origins show diverse chemical compositions and bioactivity. Therefore, discovering new plant-based anticancer agents from other parts is always challenging (Khan et al., 2022).

In this study, two compounds were isolated, namely (1) Stigmasta-5,22-dien-3-ol (STML) and (2) 1,2-Benzene dicarboxylic acid, 1,2-bis (2-Ethylhexyl) esters (DEHP) using 1D, 2D NMR, and mass spectrometry. Meanwhile, the activity of CRH was tested against several cancer cell pathways using the SRB method. These compounds have never been reported or isolated from this plant.

## Materials and Methods

### Plant material

The CRH was obtained from Makassar City, Tamalanrea Sub-District, South Sulawesi Province, Indonesia. The plant identification was conducted at the Botanical Laboratory in the Department of Biology, Mathematics, and Natural Sciences, Universitas Negeri Makassar. Furthermore, the plant samples were washed with running water, wet sorted, cut into small pieces, dried in a 40-50 °C oven for three days, dry sorted, and stored in containers tightly sealed with silica gel.

### Extraction and isolation

The extraction of *Cleome rutidospermae* herb (2 kg) with hexane solvent (1:10 ml) was conducted using the maceration method at room temperature for three days (Tiwari et al., 2011; Yasir et al., 2022). It was stirred once daily and filtered using a vacuum pump with whatmanTM no 42-filter paper. Subsequently, the liquid extract was evaporated to obtain a 35 g viscous extract using rotavapor and a water bath. The extract was partitioned using petroleum ether and methanol (1:1) three times as much as 250 ml. The petroleum ether and methanol extracts obtained were 29.2 g and 5.74 g, respectively.

The methanol extract was partitioned with hexane and methanol-water comparison (1:1) 3 times as much as 300 ml to obtain methanol-water and hexane partitioned extract of 0.4753 g and 5.3247 g, respectively. The hexane partitioned extract was isolated using a normal phase (NP) silica chromatography column, with hexane and ethyl acetate mobile phase (3:1) 3 times as much as 300 ml. The partitioned using 200 ml ethyl acetate and 200 ml methanol to give 1-5 sub-fraction, sub-fraction (2) 0.0514 g washed with hexane, and insoluble hexane (b) 0.0230 g. Subsequently, the soluble hexane (a) 0.0284 g was isolated using an NP silica chromatography column with hexane mobile phase and ethyl acetate (4:1). This aims to obtain (1) STML 0.0202 g amorphous powder. Sub-fraction (3) 0.0644 g was washed with acetone to obtain soluble and insoluble acetone of (b) 0.0086 g and (a) 0.0438 g, respectively. The insoluble acetone was isolated using PTLC, NP silica with hexane mobile phase, and ethyl acetate (5:1) to obtain (2) DEHP 0.0335 g yellowish oil compound.

### Cytotoxicity activity

The samples of CRH weighed as much as 1 mg with a 20 µg/ml concentration. The test used five lines

of human tumor cells, namely A549 (lung carcinoma), MDA-MB-231 (estrogen receptor-negative, progesterone receptor-negative, and HER2-negative breast cancer), MCF-7 (estrogen receptor-positive, HER2-negative breast cancer), KB (isolated initially from epidermoid carcinoma of the nasopharynx), and KB-VIN (vincristine (VIN)-resistant KB subline showing MDR phenotype by overexpressing P-gp). All the cell lines were obtained from Lineberger Comprehensive Cancer Center (UNC-CH) or ATCC. Furthermore, 4,000–12,000 cells were seeded in the microtiter plate of 96 wells, with each test sample in DMSO (negative control). Subsequently, 10% trichloroacetic acid was added after 72 hours, followed by the 0.04% sulforhodamine B method. DMSO substances with 0.1% v/v concentration showed no inhibitory effect on cancer cells (Rahim et al., 2018). The growth (%) was calculated on a plate-by-plate basis for tested wells compared to the control. It was also articulated using the following formula, Average absorbance of test wells  $\times$  100 / Average absorbance of control wells (Houghton et al., 2007; Lamkanfi et al., 2008).

## Results

### Isolation compound

The CRH was checked profile using <sup>1</sup>H NMR (Figure 1) and contained unsaturated fatty acids, saturated fatty acids, lipids, glycerol, ω-3 fatty acids, cholesterol, and position of functional groups in Table 1. It was partitioned using petroleum ether, and MeOH with the ratio of (1:1). The soluble layer of MeOH was evaporated and further partitioned into a combination of hexane, MeOH-H<sub>2</sub>O (1:1) as much as 300 ml. A combination of various chromatography techniques was also used to separate the hexane layer from obtaining the following compounds (1) STML and (2) DEHP with reported 1D spectroscopic, 2D NMR, and mass spectrometry data. The STML (1) is a white amorphous powder and steroid group compound based on ESI+ MS m/z 413.18 [M + H]<sup>+</sup> spectrometry mass data with the molecular formula C<sub>29</sub>H<sub>48</sub>O Figure 3. According to the <sup>1</sup>H NMR spectroscopy in Table 2 showing the presence of 5 methyl group signals, the proton methine, which appeared at position δH 5.35 (1H, *br d*, *J*=5.50 Hz), δH 5.17 (1H, *dd*, *J*=8.24 Hz), δH 4.99 (1H, *dd*, *J*=8.70 Hz), and δH 3.52 (1H, *septet*) were emerging hydroxyl group signal positions or methylene protons. That is observed in carbon positions using DEPT 135 data. Furthermore, the <sup>13</sup>C NMR showed the compound had 29 carbons, and DEPT 135 data indicated the function of carbon with 5 protons methyl and methylene group. Meanwhile, the carbon position of methine was δC 71.82, which binds to hydroxyl groups, δC 121.72, δC 138.32, δC 129.27 as a double bond, and carbon without protons at position δC 140.75, δC 36.51, and δC 42.21.

NMR's 2-dimensional data, including HMQC and HMBC, showed the correlation between hydrogen and carbon in Figure 2, blue line, i.e., methyl proton δH 1.02 (3H, *d*, *J* = 7.79 Hz) correlated with carbon δC 36.51 (C-10), δC 37.25 (C-1), δC 50.15 (C-15 9), and δC 140.75 (C-5). Proton methyl δH 1.03 (3H, *s*) correlated with δC

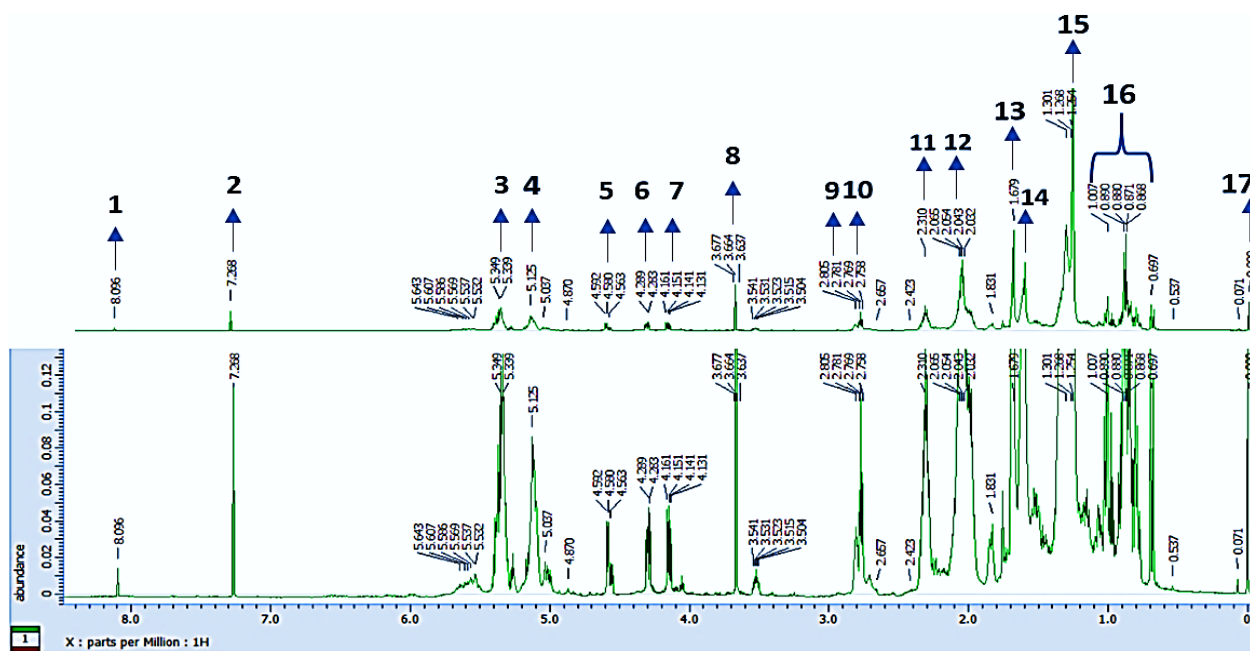


Figure 1. <sup>1</sup>H NMR Spectra of *Cleome Rutidospermae* Herb Hexane Extract (CRH). Assignments (A) 1 to 17 are reported in table 1 (400 MHz, CDC13). (A) = Major signal; (B) = (A) An expanded

40.51 (C-20),  $\delta$ C 55.94 (C-17), and  $\delta$ C 138.32 (C-22). Furthermore,  $\delta$ H 0.68 (3H, m) correlated with  $\delta$ C 55.94 (C-17),  $\delta$ C 42.21 (C-13), and  $\delta$ C 39.77 (C-12), while  $\delta$ H 0.80,  $\delta$ C 11.87 (3H, m) correlated with carbon  $\delta$ C 18.98 (C-28) and  $\delta$ C 51.24 (C-24). In addition,  $\delta$ H 0.80,  $\delta$ C 12.05 (3H, m) correlated with  $\delta$ C 39.68 (C-25) and  $\delta$ C 51.24 (C-24).

The correlation data with protons using 1H-1H Cosy in figure 2, the thick red line showed proton H3  $\delta$ H 3.52 (1H, septet) correlated with H2 and H4, H6  $\delta$ H 5.35 (1H, *br d*,  $J=5.50$  Hz) with H7, H22  $\delta$ H 5.17 (1H, *dd*,  $J=8.24$  Hz) correlated with H20 and H23  $\delta$ H 4.99 (1H, *dd*,  $J=8.70$  Hz) and proton H24. Furthermore, 1D and 2D NMR data

indicated that this compound first reported in the plant was steroid (1) STML.

The DEHP (2) is a yellow oil and phthalate group compound. According to spectrometry, mass data gave a molecular weight of ESI+ MS  $m/z$  391.20 [M + H]<sup>+</sup> with the C<sub>24</sub>H<sub>38</sub>O<sub>4</sub> formula Figure 3. The same compound was previously isolated from Aloe vera plants and discovered to have antileukemic as well as antimutagenic effects (Lee et al., 2010). The <sup>1</sup>H NMR spectroscopy data in Table 2 showed the presence of 4 methyl group signals, ten methylene protons, and four methines observed at the proton position using DEPT 135 and HMBC data. According to <sup>13</sup>C NMR, this compound has 24 carbons,

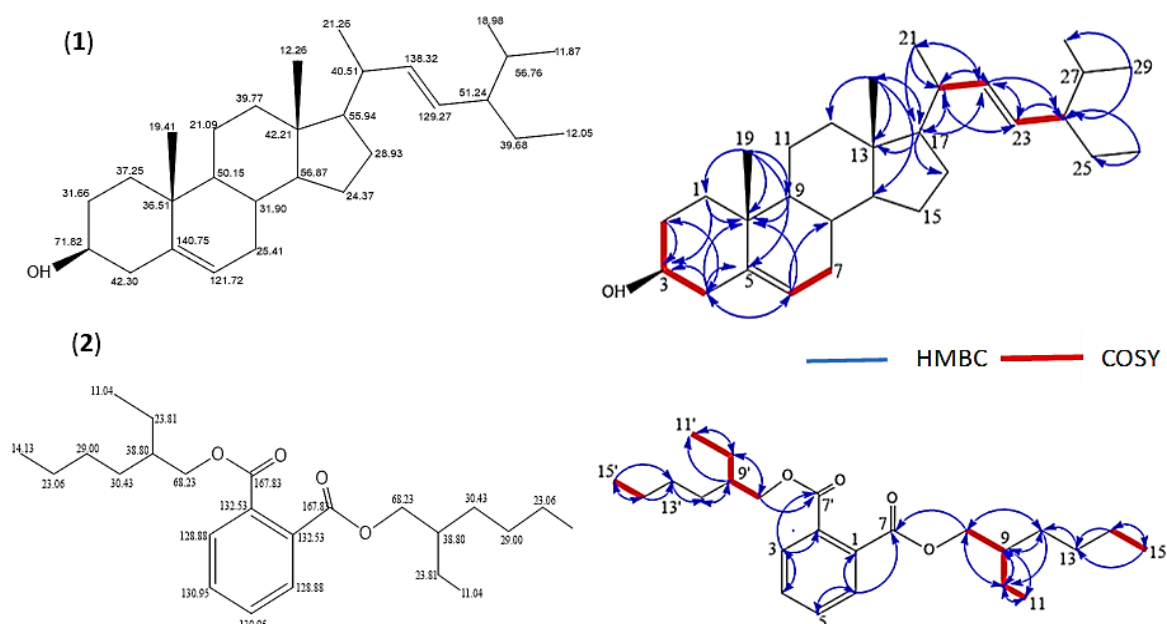


Figure 2. Structure of Compound (1) Stigmasta-5,22-dien-3-ol (STML) and (2) 1,2-Benzene dicarboxylic acid, 1,2-bis(2-Ethylhexyl) ester (DEHP)

Table 1. <sup>1</sup>H NMR Spectra Signal from Cleome Rutidospermae Herb Hexane Extract (CRH) Assignments (A) 1 to 17

Peak (A)	$\delta$ (ppm)	Multiplicity	Compound/proton	References $\delta$ (ppm)
1	8.1	<i>s</i>	Trans olefinic	8.1, <i>d</i> (Hasan et al., 2007)
2	7.24	<i>s</i>	Chloroform (solvent)/(CHCl <sub>3</sub> )	7.26 (de Combarieu et al., 2015; Gottlieb et al., 1997)
3	5.37-5.30	<i>m</i>	All unsaturated fatty acids/(CH = CH) Methylene protons/(CH <sub>2</sub> )	5.36 (de Combarieu et al., 2015) 5.37 – 5.30, <i>m</i> 2H (Shirey et al., 2018)
4	5.12-5.07	<i>m</i>	Glycerol (triacylglycerols)/CHOCOR, Glycerol (sn-1,2-diacylglycerols)/CHOCOR Methinic (CH), Aliphatic/(CH <sub>2</sub> )	5.27; 5.08 (de Combarieu et al., 2015) 5.12; 5.07 (Que et al., 2000; Sivakumar et al., 2013)
5	4.57	<i>d</i> , <i>J</i> =6.9 Hz	Glycerol (all acylglycerols)/CH <sub>2</sub> OCOR	4.30 (de Combarieu et al., 2015)
6	4.28	<i>dd</i> , <i>J</i> =11.9, 4.3 Hz	Vinyl protons Glycerol (all acylglycerols)/CH <sub>2</sub> OCOR	4.28 ( <i>d</i> , <i>J</i> = 3.2 Hz) (Breen et al., 2005) 4.15 (de Combarieu et al., 2015)
7	4.13	<i>q</i> , <i>J</i> =6.0 Hz	Glycerol (sn-1,3-diacylglycerols)/CHOH Methylene group/(CH <sub>2</sub> O)	4.07 (de Combarieu et al., 2015) 4.13; <i>q</i> , <i>J</i> = 6 Hz (Reheim & Tolba, 2016)
8	3.64	<i>s</i>	Fatty alcohols/(CH <sub>2</sub> OH) Polyethylene glycol/(OCH <sub>2</sub> CH <sub>2</sub> )	3.64 (de Combarieu et al., 2015) 3.64, <i>s</i> (Fang et al., 2014)
9	2.78	<i>t</i>	Linoleyl and linolenyl/(CH=CHCH <sub>2</sub> CH = CH) Methylene/(CH <sub>2</sub> )	2.81 (de Combarieu et al., 2015) 2.78, <i>t</i> (Flores et al., 2013)
10	2.74-2.79	<i>t</i> , <i>m</i>	$\omega$ -3 fatty acids, Ethylene (CH <sub>2</sub> CH <sub>2</sub> )	2.74, <i>t</i> ; 2.80–2.73, <i>m</i> (Li et al., 2018; Vicente et al., 2015)
11	2.25	<i>m</i>	Methylenic/(CH <sub>2</sub> )	2.25 (Durand et al., 2020)
12	1.94-2.09	<i>m</i>	Allylic (CHCHCH <sub>2</sub> )	1.94–2.15 (Hwang et al., 2017)
13	1.65	<i>s</i>	All acyl chains/(CH <sub>2</sub> CH <sub>2</sub> COOH) Methylene/(CH <sub>2</sub> )	1.64 (de Combarieu et al., 2015) 1.65 (Tshilanda et al., 2014)
14	1.57-1.58	<i>s-m</i>	Lipids/(CH <sub>2</sub> CCO)	1.58 (Righi et al., 2014)
15	1.2-1.4	<i>m</i>	Saturated fatty acids/(CH) <sub>n</sub>	1.2-1.4, <i>m</i> (Knothe & Kenar, 2004)
16	1.83-0.66	<i>m</i>	Cholesterol and methyl groups/(CH <sub>3</sub> )	0.66; 1.83 (Park et al., 2011; J. Zhao et al., 2011)
17	0	<i>s</i>	Tetramethylsilane/(TMS)	0.02, <i>s</i> (Hoffman, 2006)

while DEPT 135 data showed a carbon position with two methyl protons at  $\delta$ C 14.13 and  $\delta$ C 11.04, respectively, with methylene carbon at  $\delta$ C 23.06,  $\delta$ C 29.00,  $\delta$ C 39.43,

$\delta$ C 23.81. Each of the four methylene protons can be observed at HMQC and HMBC positions, and the 2 proton acetolactone position  $\delta$ C 68.23 (CH<sub>2</sub>OCO). Furthermore,

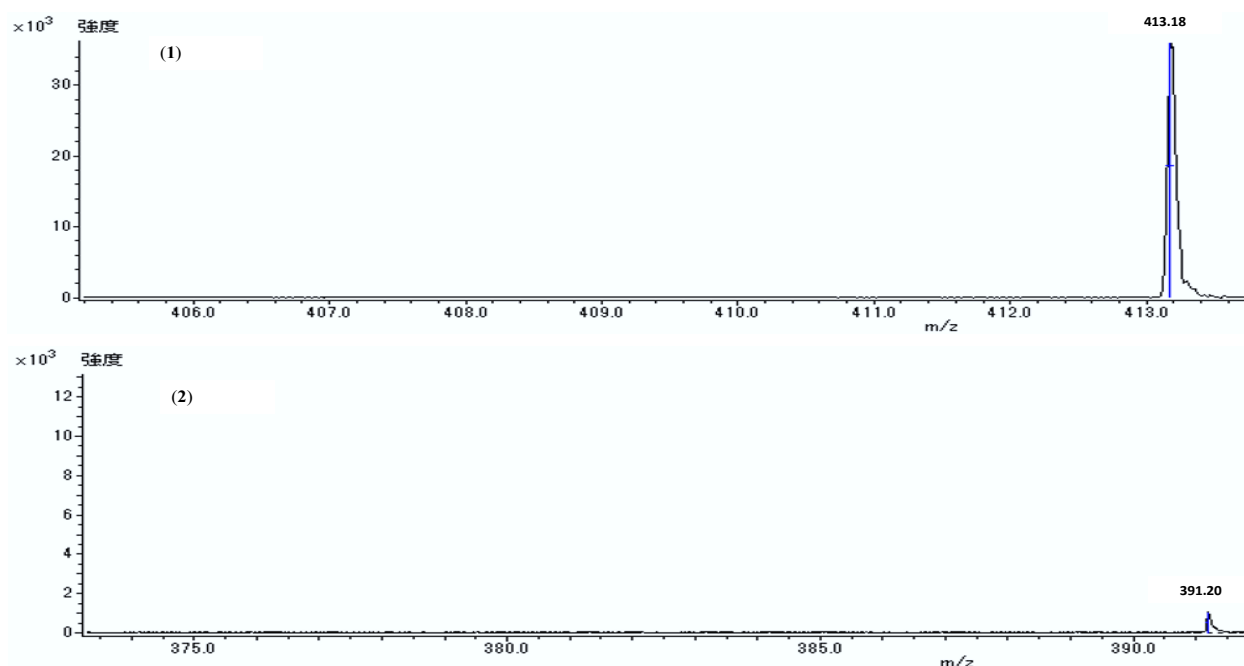


Figure 3. Data Mass Spectra of (1) Stigmasta-5,22-dien-3-ol (STML) ESI+  $m/z$  413.18 [M + H]<sup>+</sup> and (2) 1,2-Benzene dicarboxylic acid, 1,2-bis (2-Ethylhexyl) ester (DEHP)  $m/z$  391.20 [M + H]<sup>+</sup> using Electrospray Ionization (ESI) mass spectrometry.

Table 2. Data <sup>1</sup>H NMR, <sup>13</sup>C NMR, and DEPT 135 of (1) Stigmasta-5,22-dien-3-ol (STML) and (2) 1,2-Benzene dicarboxylic acid, 1,2-bis (2-Ethylhexyl) ester (DEHP)

STML (1)				DEHP (2)			
Position	DEPT	<sup>13</sup> C (ppm)	<sup>1</sup> H (ppm)	Position	DEPT	<sup>13</sup> C (ppm)	<sup>1</sup> H (ppm)
1	CH <sub>2</sub>	37.25	<i>br d</i> , 5.35 (1H) <i>J</i> =5.50 Hz	1	C	132.53	<i>septet</i> , 3.51 (1H)
2	CH <sub>2</sub>	31.66	<i>dd</i> , 5.17 (1H) <i>J</i> =8,24 Hz	2	C	132.53	<i>br d</i> , 5.33 (1H)
3	CH	71.82	<i>dd</i> , 4.99 (1H) <i>J</i> =8,70 Hz	3	CH	128.88	<i>dd</i> , 5.22 (1H)
4	CH <sub>2</sub>	42.30	<i>septet</i> , 3.52 (1H)	4	CH	130.95	<i>dd</i> , 5.16 (1H)
5	C	140.75	<i>m</i> , 0.82 (3H)	5	CH	130.95	<i>br q</i> , 2.42 (1H)
6	CH	121.72	<i>m</i> , 0.82 (3H)	6	CH	128.88	<i>br s</i> , 4.68 (2H)
7	CH <sub>2</sub>	25.41	<i>d</i> , 1.02 (3H) <i>J</i> =7,79	7	C	167.83	
8	CH	31S.90	<i>s</i> , 1.03 (3H)	8	CH <sub>2</sub>	68.23	
9	CH	50.15	<i>m</i> , 0.80 (3H)	9	CH	38.8	
10	C	36.51	<i>m</i> , 0.68 (3H)	10	CH <sub>2</sub>	23.81	
11	CH <sub>2</sub>	21.09		11	CH <sub>3</sub>	11.04	
12	CH <sub>2</sub>	39.77		12	CH <sub>2</sub>	30.43	
13	C	42.21		13	CH <sub>2</sub>	29	
14	CH	56.87		14	CH <sub>2</sub>	23.06	
15	CH <sub>2</sub>	24.37		15	CH <sub>3</sub>	14.13	
16	CH <sub>2</sub>	28.93					
17	CH	55.94					
18	CH <sub>3</sub>	12.26					
19	CH <sub>3</sub>	19.41					
20	CH	40.51					
21	CH <sub>3</sub>	21.26					
22	CH	138.32					
23	CH	129.27					
24	CH	51.24					
25	CH <sub>2</sub>	39.68					
27	CH	56.76					
28	CH <sub>3</sub>	18.98					
26	CH <sub>3</sub>	12.05					
29	CH <sub>3</sub>	11.87					

the part of two carbon methines on the benzene framework is located at  $\delta$ C 128.88 and  $\delta$ C 130.95 and had a proton correlation with carbon at  $\delta$ C 130.95.

NMR's 2-dimensional data including HMQC and HMBC showed the correlation between hydrogen and carbon at (Figure 2, blue line) i.e. 2 methyl protons  $\delta$ H 0.90 (3H, m) (C-15, C-15') correlated with carbon  $\delta$ C 23.06 (C-14, C-14'), and  $\delta$ C 29.00 (C-13, C-13'). Also, 0.92 (3H, s) (C-11, C-11') correlated with  $\delta$ C 23.81 (C-10, C-10'), and  $\delta$ C 38.80 (C-9, C-9'),  $\delta$ H 4.22 (2H, n) (C-8) with carbon carbonyl position  $\delta$ C 167.83 (C-7, C-7'). Furthermore, two protons methine  $\delta$ H 1.42 (1H, m) (C-9, C-9') correlated with carbon (C-11, C-11', C-10, C-10', C-12, C-12'), methylene protons  $\delta$ H 1.32 (2H, m) (C-10, C-10') correlated with carbon  $\delta$ C 38.80 (C-9, C-9') and  $\delta$ C 23.81 (C-10, C-10').  $\delta$ H 1.33 (2H, m) (C-13, C-13') correlated with  $\delta$ C 30.41 (C-12, C-12'), and two methylene protons 1.30 (2H, m) (C-14, C-14') correlated with  $\delta$ C 29.00 (C-13, C-13') and  $\delta$ C 14.13 (C-15, C-15'). In addition, the proton methine H-3  $\delta$ H 7.70 (1H, m)

correlated with carbon  $\delta$ C 130.95 (C-4),  $\delta$ C 132.53 (C-2), and methine H-6  $\delta$ H 7.70 (1H, m) with carbon  $\delta$ C 130.95 (C-5), and  $\delta$ C 132.53 (C-1).

The correlation data with protons using 1H-1H Cosy (Figure 2, thick red line) showed that proton methyl H-15, H-15'  $\delta$ H 0.90 (3H, m) correlated with H-14, H-14'. Furthermore, H-11, H-11' 0.92 (3H, s) correlated with H-10, H-10'  $\delta$ H 1.32 (2H, m), while H-10, H-10' correlated with methine H-9, H-9'  $\delta$ H 1.42 (1H, m), and proton H-9, H-9' with H-8, H-8'  $\delta$ H 4.22 (2H, n).

Also, 1D and 2D NMR data indicated that this compound was a group of phthalate acid (2) DEHP and was first reported in this plant. In addition, it was isolated from *Streptomyces bangladeshensis* sp. Nov (Al-Bari et al., 2005), *Aspergillus awamori* (Lotfy et al., 2018), Aloe vera (Lee et al., 2010), *Calotropis gigantea* flower (Linn.) (Habib and Karim, 2009), *Penicillium janthinellum* (El-sayed et al., 2015), *Acinetobacter* sp. SN13 (Xu et al., 2017), and *Benincasa hispida* flower (Du et al., 2006).

### Cytotoxic study

The preliminary test results of the CRH against cytotoxic cancer cells with 20 µg/mL showed that the % growth of MCF-7 cancer cells was 28.1%, A549 at 60.6%, and MDA-MB-231 at 68.4%. Furthermore, KB 58.1% and KB-VIN 86.3%, compared to the negative DMSO of 0.20%, control the growth of all cancer cells (100%).

The inhibition of these cells correlated with the contents which were successfully isolated in CRH, such as (1) STML that have inhibition of cancer cells >50% in ES2 and OV90 (Bae et al., 2020), SGC-7901, and MGC-803 (Zhao et al., 2021), as well as MCF-7 and NIH/3T3 cancer cells (Ayaz et al., 2019), which are chemopreventive (Ali et al., 2015). Also, (2) DEHP is effective as an anticancer. Therefore, as the compound concentrations increase, it inhibits the growth of cancer cells MCF-7, HEPG2, HELA, and HCT116 (Lotfy et al., 2018). This compound has potent cytotoxicity for MCF-7 cancer cells with IC<sub>50</sub> of 6.941 µg/ml, moderate cytotoxicity for a HEPG2 cancer cell, and IC<sub>50</sub> of 26.73 µg/ml for HELA cancer cell and showed no activity against colon carcinoma (HCT116) (Lotfy et al., 2018). Subsequently, it inhibits basal alveolar epithelial cells A-549 370 µg/mL (El-sayed et al., 2015).

### Discussion

Many studies have been published regarding the anticancer potential of various plant extracts that can be good candidates for cancer drug discovery and development one example was the paclitaxel compound isolated from *Taxus brevifolia* for chemotherapy use (Liebmann et al., 1993). This STML (1) was first reported to be found in this species. However, this compound has been reported in several species of Cleome. STML, also known as stigmaterol once isolated from the *Cleome gynandra* (Ranjitha et al., 2009), *Cleome paradoxa* (Abdel-Monem, 2012), and *Cleome viscosa*. It was reported that STML compounds have the potential to be anticancer for new treatments in ovarian cancer (Bae et al., 2020), gastric cancer (Zhao et al., 2021), glycolysis in triple-negative breast cancer cells (MDA-MB-231), and lung cancer (Dong et al., 2021). This compound has an anticancer mechanism through the induction of cell apoptosis, ROS production, and excess calcium in ES2 and OV90 cells. STML stimulates cell death by activating ER-mitochondria, suppressing cell migration and angiogenesis genes in human ovarian cancer cells (Bae et al., 2020). In addition, this compound also induces apoptosis and autophagy by inhibiting the Akt/mTOR pathway in gastric cancer cells (Zhao et al., 2021) and regulating retinoic acid associated with orphan C receptors (Dong et al., 2021).

The DEHP (2) compound is one of the compounds successfully isolated in this plant and first reported in this species. This compound has been reported to be found in *Cleome serrata* (Mcneil et al., 2012). In addition, compounds 2-ethyl-cyclohexane-2-ene-6-hydroxy-methylene-1- carboxylic acid and 3β-hydroxy-lup-20(29)-en-28-oic acid were also found in this plant (Rahman et al., 2008). DEHP (2) is known to have the potential to be an anticancer in the treatment of cancers such as breast

cancer, liver cancer, cervical cancer, and colon cancer (Lotfy et al., 2018). Compound Bisphenol A combination with DEHP mainly induced the expression of HDAC6, inhibited tumor suppressor gene PTEN upregulated the expression of oncogene c-MYC. Eventually, it elevated the susceptibility to thyroid tumors (Zhang et al., 2022).

### Author Contribution Statement

BY and AR conceptualized and designed the study; BY performed the experiments and collected the data; S, YS, KNG, and BY analyzed and interpreted the results; BY prepared the initial manuscript; AR and GM reviewed and approved the final version of the manuscript.

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### Statement of conflict Interest

The authors do not have any conflicts of interest to declare.

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