© 2021 Journal of Pharmacy & Pharmacognosy Research, 9 (3), 344-356, 2021
ISSN 0719-4250
http://jppres.com/jppres

Original Article

The chemical composition of the ethanolic extract from *Chromolaena odorata* leaves correlates with the cytotoxicity exhibited against colorectal and breast cancer cell lines

[La composición química del extracto etanólico de las hojas de *Chromolaena odorata* se correlaciona con la citotoxicidad exibida contra líneas celulares de cáncer colorrectal y de mama]

Hanifah Yusuf[1*], Fauzul Husna[1], Basri A. Gani[2]

[1]Pharmacology Department, Faculty of Medicine, Universitas Syiah Kuala, Jl. Teuku Tanoh Abee, Kopelma Darussalam, Syiah Kuala, Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Aceh 23111. Indonesia.
[2]Oral Biology Department, Faculty of Dentistry, Universitas Syiah Kuala, Jl. Teuku Nyak Arief No. 441, Kopelma Darussalam, Kecamatan Syiah Kuala, Kota Banda Aceh, Aceh 23111. Indonesia.
*E-mail: hans_yusuf1104@unsyiah.ac.id

Abstract

Context: Colorectal and breast cancer are of particular cellular preventive concern. WIDR cells are an indicator of colorectal cancer development, while HTB and 4T1 cells are involved in breast cancer. The *Chromolaena odorata* was reported to interfere with the growth of the three cancer cells.

Aims: To evaluate the bioactivity of *C. odorata* against the cytotoxicity of colorectal and breast cancer cells.

Methods: Ethanol extract of *C. odorata* leaves was examined to find chemical compounds by GC-MS, quality of antioxidants by DPPH assay, and antioxidants quantity by flavonoids and phenolic assay, whereas ionic values were evaluated by the multi-tester meter also cytotoxicity on the WiDr, HTB, and 4T1 cancer cells by MTT assay.

Results: *C. odorata* contained six antioxidant compounds with quality above 80%. Alpha-amyrin has the most retention time of 38.112 min. The *C.odorata* had an antioxidant rate (IC_{50}) higher than vitamin C. Total flavonoids (72%) higher than total phenolic (28%), and both have a significant impact on the WiDr, HTB, and 4T1 cancer cells ($p<0.05$). The value of ionic dissolved oxygen, conductivity, and total dissolved solids was 33%, respectively ($p>0.05$). The *C. odorata* had a higher cytotoxicity effect on the 4TI and HTB cells in breast cancer than WiDr cancer cells in colorectal cancer.

Conclusions: The *C. odorata* has significant antioxidant quality and quantity with a stable ionic value also has a cytotoxic effect on the WIDR, HTB, and 4T1 cancer cells.

Keywords: antioxidant; breast cancer; *Chromolaena odorata*; colorectal cancer; cytotoxicity; ionic value.

Resumen

Contexto: El cáncer colorrectal y de mama son de particular interés en la prevención celular. Las células WIDR son un indicador del desarrollo del cáncer colorrectal, mientras que las células HTB y 4T1 están involucradas en el cáncer de mama. Se informó que la *Chromolaena odorata* interfiere con el crecimiento de las tres células cancerosas.

Objetivos: Evaluar la asociación de la bioactividad de *C. odorata* con las células de cáncer de mama y colorrectal.

Métodos: Se examinó la composición química del extracto etanólico de hojas de *C. odorata* por GC-MS, calidad de antioxidantes por ensayo de DPPH y cantidad de antioxidantes por flavonoides y ensayo fenólico, mientras que los valores iónicos fueron evaluados por el multímetro y citotoxicidad en el WiDr, HTB y células cancerosas 4T1 mediante ensayo MTT.

Resultados: *C. odorata* contenía seis compuestos antioxidantes con calidad superior al 80%. La alfa-amirina tuvo el mayor tiempo de retención de 38,112 min. La *C. odorata* tuvo una tasa de antioxidantes (IC_{50}) más alta que la vitamina C. Flavonoides totales (72%) más altos que los fenólicos totales (28%), y ambos tuvieron un impacto significativo en las células cancerosas WiDr, HTB y 4T1 ($p<0,05$). El valor de oxígeno iónico disuelto, conductividad y sólidos disueltos totales fue 33%, respectivamente ($p>0.05$). *C. odorata* tuvo un mayor efecto de citotoxicidad sobre las células 4TI y HTB en el cáncer de mama que las células cancerosas WiDr en el cáncer colorrectal.

Conclusiones: La *C. odorata* tiene una cantidad y calidad antioxidante significativa con un valor iónico estable que también tiene un efecto citotóxico sobre las células cancerosas WIDR, HTB y 4T1.

Palabras Clave: antioxidante; cáncer de mama; *Chromolaena odorata*; cáncer colon; cáncer rectal; citotoxicidad; valor iónico.

ARTICLE INFO
Received: November 1, 2020.
Received in revised form: December 12, 2020.
Accepted: December 14, 2020.
Available Online: January 15, 2021.

INTRODUCTION

Cancer is reportedly the second leading cause of death globally. About 70% of cancer deaths occur in low and middle-income countries. Cancer deaths continue to increase, with an estimated 13.1 million people in 2030. Colorectal cancer causes 862 000 deaths per year, while breast cancer is around 627 000 per year. In general, two factors cause cancer, namely, internal factors (heredity), and external factors (hormonal changes, obesity, smoking habits, exposure to radiation, viruses, and chemicals). The spread of cancer depends on the type and location of the tumor. In general, about 90-95% of cancer cases are caused by genetic mutations influenced by environmental and lifestyle factors. It is estimated that the remaining 5-10% of hereditary genetics factor (WHO, 2018). These factors work separately or together in causing colorectal cancer and breast cancer (Abdulkareem, 2013). Colorectal cancer (CRC) is part of the development of colon cancer. WiDr cells are reported as an indicator of colon tumor biology. WiDr cell proliferation is triggered by HT-29 cells by expressing cycloxygenase-2 (COX-2) (Rajamanickam and Agarwal, 2008). WiDr cell abnormality occurs due to p53 G mutation. Still, it can be done on the independent p53 pathway activate p73 to help WiDr cell apoptosis.

Meanwhile, p21 in normal WiDr cells still functions as a checkpoint for the cell cycle (Aubrey et al., 2018). Besides, breast cancer is mostly found in tissues, mammary ducts formed in fatty tissue, or connective tissue in the breast (Kamarlis et al., 2017). The cells mammary adenocarcinoma murine cells can reference breast cancer cells to represent the subtype of triple-negative breast cancer (TNBC). At the same time, the HTB cancer cells are induced by adipocyte lipolysis in obesity factors. Fatty acids that facilitated breast cancer growth are derived from adipocytes that promote breast cancer cells' proliferation and migration (Balaban et al., 2017).

WiDr, 4T1, and HTB cancer cells substantially affect increasing cancer spread at colon and breast sites. Various strategies are studied to prevent cancer metastases, including both of these cancers. This action of eliminating cancer cells becomes a strategy to avoid the causes of these two cancers, such as radiation therapy, hormone therapy, and chemotherapy. The goal of this treatment is to prevent the development of cancer cells. Besides, cancer surgical treatment aims to remove tumor cells and symptoms. This strategy is considered unable to prevent the spread of cancer cells because, at any time, cancer cells can mutate genes to form new cancer cells. The prevention of cancer cells has been studied *in vivo* and *in vitro*; therapy of natural plants' use has become a trend by world pharmacologists to find toxic materials to prevent cancer cells' development. These strategies can repair DNA and RNA damaged during gene mutations through signaling in the cell cycle pathways (Wang et al., 2012).

Chromolaena odorata (L.) R.M.King & H.Rob. (*Compositae*) exhibits anti-cancer and wound healing of skin infections (Sirinthipaporn and Jiraungkoorskul, 2017). Also, *C. odorata* is anticlonogenic against breast cancer cells type Ca151 by inhibiting BcL2 ABT737 (Kouamé et al., 2013). In contrast, *C. odorata* is sensitive to breast cancer cells MCF-7 and BT-20 and HT-29 cell lines (Adedapo et al., 2016). This study evaluated the cytotoxicity properties of *C. odorata* towards WiDr cells in colon cancer and HTB and 4T1 cells in breast cancer.

MATERIAL AND METHODS

The research approved ethical clearance from the Ethics Commission, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, Indonesia No. 239/KEPK/FK UNLAM/VI/2020. This study used the ethanol extract of *C. odorata* and WiDr cancer cells (ATCC® CCL-218 ™), colon cancer cells. BT-549 (ATCC® HTB-122 ™) and 4T1 (ATCC® CRL2539 ™) of breast cancer cells.

Plant material

The plant was obtained from Aceh Besar District, Aceh Province, Indonesia (5.603444, 95.405863). The ethanol extract of *C. odorata* was

obtained from the Chemical Laboratory, Faculty of Mathematics and Natural Science, Universitas Syiah Kuala, Darussalam Banda Aceh Indonesia. Voucher Number Co1820. Its assay material was collected in Pharmacology Laboratory, Medicine Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Indonesia.

Phytochemical screening

A total of 500 mg of ethanol extract was dissolved in 50 mL of 96% ethanol. Qualitative analysis was carried out as followed: (1) Alkaloid test: 2 mL of the test solution was evaporated on a porcelain dish until obtained a residue. The residue was dissolved with 5 mL of 2 N HCl. The solution obtained was divided into 3 test tubes. To the first tube was added dilute acid (blank). The second tube added three drops of Dragendroff reagent, and the third tube was added three drops of Mayer's reagent. Orange deposits in the second tube and yellow deposits in the third tube indicate the presence of alkaloids. (2) Test steroids and triterpenoids: a total of 2 mL of the test solution was evaporated in a Petri dish. The residue formed was dissolved with 0.5 mL of chloroform, then 0.5 mL of anhydrous acetic acid, and 2 mL of concentrated sulfuric acid. Brownish or violet rings on the solution's border indicated triterpenoids, whereas if a bluish-green ring appears, it showed steroids. (3) Saponin test: as much as 10 mL of the test solution was added to the test tube, shaken vertically for 10 seconds, then left for 10 seconds. The formation of foam as high as 1-10 cm, which persisted for about 10 min, indicated saponins' presence, and if a drop of 2 N HCl was added, the foam does not disappear. (4) Test for tannins and polyphenols: 2 mL of the test solution divided into two different tubes. The tube was used as a blank and reacted with 10% iron (III) chloride solution. Dark blue or greenish-black color indicated the presence of tannins and polyphenols, and (5) flavonoid test: 1 mL of test solution moistened with acetone, added a little boric acid fine powder and oxalic acid fine powder, heated on a water bath. The residue obtained was mixed with 10 mL ether and then observed with 366 nm UV light. The intensive yellow fluorescent solution indicated the presence of flavonoids (Sasidharan et al., 2011).

Extraction and Gas Chromatography-Mass Spectrometry assay

A total of 1 kg of *C. odorata* leaves were cut at 0.2 mm and then macerated in 5 L of 80% ethanol for 24 h. Furthermore, stirring every 4 h, then decanted and filtered and added 80% ethanol and soaked for 48 h. The filtrate obtained was collected and evaporated by a rotary vacuum evaporator. Then it was heated at 45°C to remove the remaining 80% ethanol and stored in a dark bottle and tightly closed. Identification of antioxidant compounds from *C. odorata* used to the Gas Chromatography-Mass Spectrometry (GC-MS) by Shimadzu QP2010PLUS, Tokyo, Japan. The FID temperature 300°C, injection temperature 220°C, the nitrogen carrier gas at a flow rate of 1 mL/min, split ratio 1:75, pressure at 116.9 kPa was carried out. The column length was 30 m with a diameter of 0.25 mm and a 50 mL/min flow rate. First, the ethanol extract of *C. odorata* was injected into the injector and evaporated. The gas carries the sample in the form of vapor to the column for the separation process. After being separated, the ionization process was carried out. The detector will capture the resulting ion fragment, and a mass spectrum is generated. After that, the data were processed to obtain chemical compounds compared with the standard reference material database. The percentage of similarity expresses the identification of analytes against the spectra standard library.

Calculation of molecular properties and bioactivity score

The mol-inspiration property engine software evaluated the calculation of bioactivity and molecular properties of *C. odorata*. Compound structures were made using Chemdraw professional v.16 © Cambridge soft software, and then the 2D designs were converted into 3D using Chem3D v.16 © Cambridge software. Furthermore, the bioactivity value and molecular score of *C. odorata* were calculated using software mol-inspiration properties engine v. 2018.10 (Alberga et al., 2018).

Total phenolics

The gallic acid standard curve determination observed the total phenolic quantity (Siddiqui et al., 2017). A volume of 1 mL of *C. odorata* in methanol was added in 0.4 mL of Folin-Ciocalteau reagent, shaken and left for 4-8 min, then added 4 mL of 7% Na_2CO_3, shaken until homogeneous. Then distilled water was add up to 10 mL and let stand for 2 h at room temperature, measured at a wavelength of 744.8 nm, performed three times was added. Phenolic content was calculated with the formula [1].

$$\text{Total phenolic} = \frac{C \times v}{m} \quad [1]$$

Where, c = gallic acid equivalent (mg/GAE/L); v = the volume of the extract solution used (mL) and m = the extract mass used (gram).

Total flavonoids

The calculation of flavonoid levels using a 0.5 mL ethanol blank consisting of 1.5 mL 95% ethanol, 0.1 mL 10% aluminum chloride ($AlCl_3$), 0.1 mL 1 M potassium acetate, and 2.8 mL distilled water, incubated for 30 min at 25°C, was performed. The determination of the total flavonoid levels of *C. odorata* was started by dissolving 100 mg of the test material in 10 mL of methanol pa. Then, take 1 mL of extract plus 10 mL of ethanol added 0.1 mL of 10% aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate, and added with distilled water up to 2.8 mM, incubated for 30 min at 25°C. It has measured at a wavelength of 434.2 nm using a UV-Vis spectrophotometer. The sample solution was made in three replications so that the levels of flavonoids obtained were equivalent to catechin. The level of flavonoids (F) was calculated using a formula [2].

$$F = \frac{C \times V \times f \times 10^2}{m} \times 100\% \quad [2]$$

Where, c = catechin equivalence (µm/mL); V = total volume of extract; f = dilution factor; and m = sample weight (g) Fattahi et al., 2014).

DPPH antioxidant assay

The examination of the antioxidant *C. odorata* used the principle of DPPH (2,2-diphenyl-1-picrylhydrazine-hydrate) (Salazar-Aranda et al., 2011). Each 100 mg of sample and vitamin C dissolved in 1 mL of DMSO and vortexed. Also, 100 µL of sample and vitamin C were included in the 96-microplate. Then added with DPPH solution of 100 µL, negative control added with ethanol p.a 100 µL. Next, incubated at room temperature for 30 min. Then read by spectrophotometry at a wavelength of 517 nm.

Ionic assay

The *C. odorata* extract's ionic examination consisted of dissolved oxygen, conductivity, and total dissolved solids (TDS). The conductivity was evaluated by conductivity meter, dissolved oxygen with a DO meter, and total dissolved solids with a TDS meter (CyberScan DO 300, Eutech Instruments Pte Ltd, Singapore) (Armstead et al., 2016). It worked based on an electrode with the probe coupled to the ampere meter voltage source and an electrode set 1 cm apart. At the time of measurement, these electrodes were immersed in the sample solution and read the values.

Cytotoxicity assay

The cells cancer culture of WIDR used CCL-218), HTB (RPMI 1640), and 4T1 were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Merck KGaA, Darmstadt, Germany). They were subsequently transferred to a culture flask, incubated in a 5% CO_2 incubator at 37°C. Confluent cell growth 70-80%., then was moved in PBS to 10 mL. Then centrifuged 3000 × g for 5 min. The cells were then washed twice, counted with a hemocytometer, and collected with a cell suspension concentration of 1×10^4 cells/100 µL. Furthermore, a stock solution of 10 mg of *C. odorata* was mixed in 100 µL of DMSO pro culture. A serial concentration of the assay solution was combined into the growth medium, then filtered with a 0.2 µm filter into a sterile conical tube. Cytotoxicity test against cancer cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) method approach assay (Abcam, Cambridge, USA). WiDr, HTB, and 4T1 cancer cells were cultured in DMEM + 10% fetal bovine serum (FBS) + 2 mM L-glutamine medium 96-well plates and then incubated in 5% CO_2 incubator for 24 h (serial dilution) with doxorubi-

cin as control. Then, added to each well plate 10 µL MTT 5 mg/mL. They have incubated again for 4 h at 37°C. Live cells will react with MTT to form a purple color. The MTT reaction was stopped with 10% SDS stopper reagent in 0.01% HCl as much as 100 µL, then incubated for 24 h at room temperature. The experiments were carried out in triplicates, and the ELISA reader read the absorbance at a wavelength of 595 nm.

Statistical analysis

The One-Way ANOVA analyzed cytotoxicity data between *C. odorata* concentrations against WIDR, HTB, and 4T1 cancer cells. Meanwhile, data on the relationship between antioxidant power, antioxidant quantity, and ionic value on cancer cell cytotoxicity were analyzed by Wilcoxon. The probability of significance was $p<0.05$ with $r = 1$ limit of correlation strength.

RESULTS

Qualitative phytochemical screening showed that the ethanol extract of *C. odorata* harbored flavonoids, steroids, quinone, polyphenol, and saponin. These findings could be the basis to suggest that *C. odorata* has anti-inflammatory and antioxidant properties, such as reported in Figs. 1-2 and Tables 1-2.

Fig. 1. shows the chromatogram of the *C. odorata* leaves extract. The GC analysis was tandem with a mass spectrometer to obtain the relative mass of each compound. The data from the instrument compared with the NIST compound database. A total of twenty-two compounds of *C. odorata* from classes were detected and tabulated in Table 1. The six major components that predict the antioxidant compound were showed as No. 5, 6, 11, 18, 19, and 22. Also, Table 1 shows that all compounds from the GC-MS examination showed excellent quality. This quality is in line with the quantity of each compound in the ethanol extract of *C. odorata*. The information from Table 1 is a reference for assessing the amount and quality of its cytotoxicity levels on the cancer cells. The compounds that represent the most antioxidants are 9,12,15-octadecatrienoic acid (12.81%) and phytol (10.46%).

Fig. 2 shows the 3D structure of 6 chemical compounds of *C. odorata*. These compounds have different bioactivity. This difference can explain that each combination has its target properties when interacting with pathogenic cells to cause cytotoxicity. The determination of bioactivity value was referred by N-Atom, molecular weight, n-ON (O-N-centered polar fragments), n-OHNH (number of hydrogen bond), and n-ROTB (number of rotatable bonds).

Table 2 shows six chemical compounds from *C. odorata* that exhibited a miLogP above 5. The vitamin E showed a higher topological polar surface area, molecular weight, and protease inhibitor than other compounds. Specifically, 9,12,15-octadecatrienoic acid had a relatively higher value of G-protein coupled receptors. Meanwhile, the ion channel modulator value of cis, cis, cis-7,10,13-hexadecatrienoic compounds was higher. In general, all these chemical compounds displayed negative kinase inhibitor values. Besides, alpha-amyrin demonstrated a higher nuclear receptor ligand and enzyme inhibitor value than other compounds.

In Fig. 3, the lowest concentration (7.8-62.5 µg/mL) showed acceptable toxicity in the presence of breast cancer cells (4T1). In contrast, the highest concentrations (125-250 µg/mL) exhibited high toxicity to breast cancer cells (HTB). However, at a 500 µg/mL concentration, it showed toxicity to all cancer cells above 80%: bar (cancer cells toxicity) and bar error (error with percentage).

Data represent means ± SD, ($n = 3$). Based on the One-Way ANOVA analysis, the concentration was a determinant of the degree of toxicity to the three cancer cells ($p<0.05$: 0.000). Meanwhile, the impact of toxicity on the three cells was not significantly different ($p>0.05$; 0.847) within the concentrations and between the three cancer cells. Pearson correlation shows a strong relationship ($r = 0.904$).

The ionic values of the ethanol extract of *C. odorata* were reported as temperature, pH, dissolved

oxygen, conductivity, and total dissolved solids. The *C. odorata* solution gave a temperature of 26.4°C with a 5.5 pH. The ionic activities measured were dissolved oxygen (6.4 DO; 33%), conductivity (196 siemens; 33%), and total dissolved solids (100 ppm; 33%). Based on the percentage value, the three ionic properties indicated a synergy when interacting with target cells. Dissolved oxygen largely determines antioxidants' work (Santos-Sánchez et al., 2019). The ionic conductivity largely circumscribes the interactions between cell receptors and the stability of the target cell surface's hydrophobicity properties (Ranke et al., 2006). Meanwhile, TDS correlates with the drug/test material's solubility when it acts as an anticancer. Its solubility relates to the material's residual, which influences the assay material's pharmacokinetic properties (Savjani et al., 2012). The effect of the three ionics from various test material concentrations on the cytotoxicity of *C. odorata* three cancer cells was no significant ($p>0.05$; 0.675).

The assessment of the free radical scavenging strength of *C. odorata* used the DPPH method. This antioxidant property is assessed based on the electron transfer, which gives a violet solution in ethanol (Maulida et al., 2019). Free radicals are stable at room temperature, reduced in antioxidant molecules, resulting in a colorless ethanol solution. The examination of the antioxidant *C. odorata* obtained very high strength (IC_{50} = 1.9 ppm) compared to the vitamin C standard (IC_{50} = 6.21 ppm). The antioxidant power at all concentrations of *C.odorata* was significantly different ($p<0.05$: 0.000).

In this study was demonstrated that the *C. odorata* extract solution contained total flavonoids (89 mg CE/g extract: 72%) and total phenolic content (34.3 mg GAE/g extract: 28%). The antioxidants composition in *C. odorata* correlates with its cytotoxicity. The Wilcoxon analysis showed that total flavonoid levels strongly influenced the cytotoxicity of *C. odorata* on the three cancer cells ($p<0.05$: 0.000). Meanwhile, there was no significant difference in phenolic total ($p>0.05$: 0.794).

Figs. 3 and 4 show that *C. odorata* exhibited a better cytotoxicity quality against breast cancer cells (HTB) compared to WiDr (colon) and 4T1 (breast) cancer cells. These figures explain each other the quantitative and qualitative phenomena to confirm that *C. odorata* presented a cytotoxicity quality of the cell cancers.

In general, *C. odorata* is highly toxic to breast cancer cells (HTB and 4T1). Meanwhile, colon cancer cells (WIDR) were less effective, except at 500 µg/mL. It assumed that cytotoxicity above 50% of *C odorata* was recommended for prevention to spread breast cancer and colon cancer.

DISCUSSION

This study evaluated the quantity and quality of antioxidants from *C. odorata* ethanol extract and the ionic value of its cytotoxicity against breast and colorectal cancer cells. Previous research studies *C. odorata* can inhibit cancer cells of mammary, tissue, and intestine (colon) (Adedapo et al., 2016). The natural materials' toxicity effect on the cancer cells is highly dependent on their bioactivity properties. The bioactivity score of a chemical compound evaluated consists of topological polar surface area, the number of rotatable bonds, ion channel modulator, a kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor) (Khan et al., 2017). G-protein largely determines the number of these factors coupled receptors when interacting with target cells (Rosenbaum et al., 2009).

The plant product has a role affects as antioxidants with stable ionic properties of solutions (Dai and Mumper, 2010). These two properties are determinants for disrupting cancer cells' surface, especially concerning the hydrophobicity, conductivity, and dissolved oxygen properties of the cell surface. The increase in these three properties can cause virulent cell surface proteins to lose receptor function, causing the cell walls' absorption and penetration to be disturbed (Campillo et al., 2019).

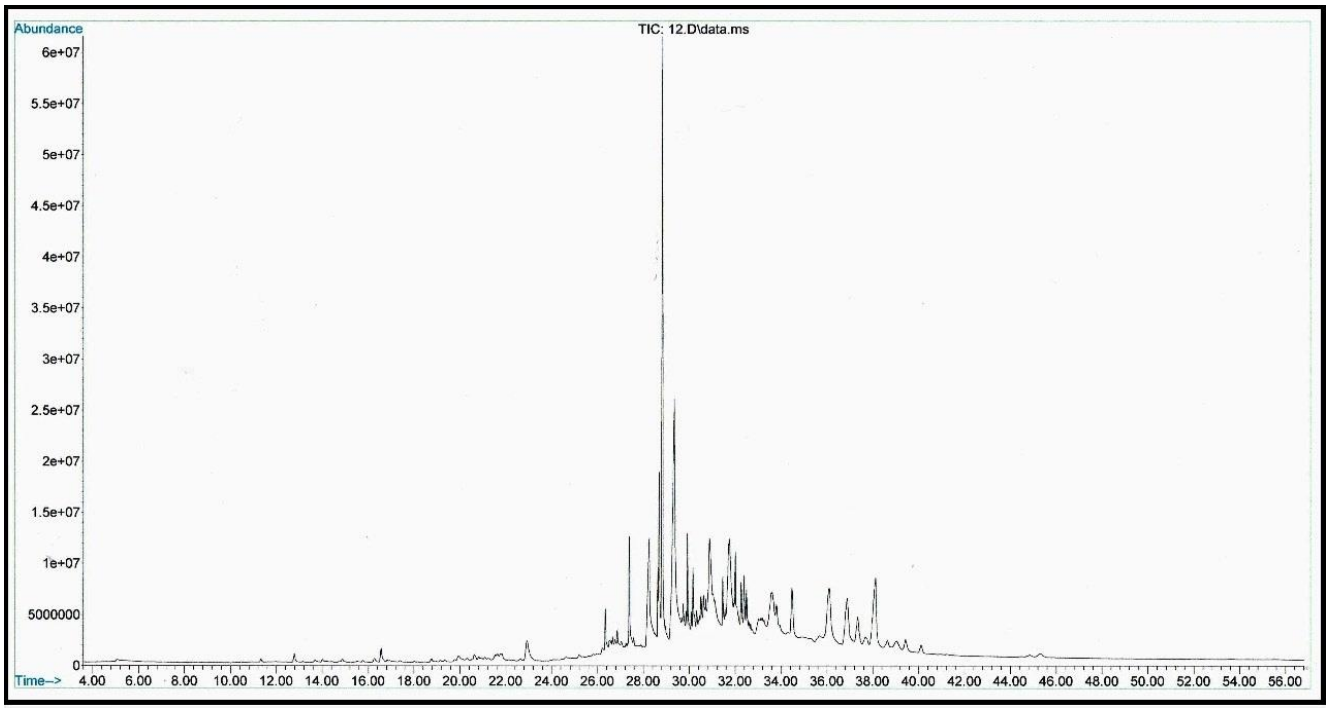


Figure 1. GC Spectrum of *C. odorata* leaves extract.
The peaks started to show up at 22.935 min of retention until 38.112 min.

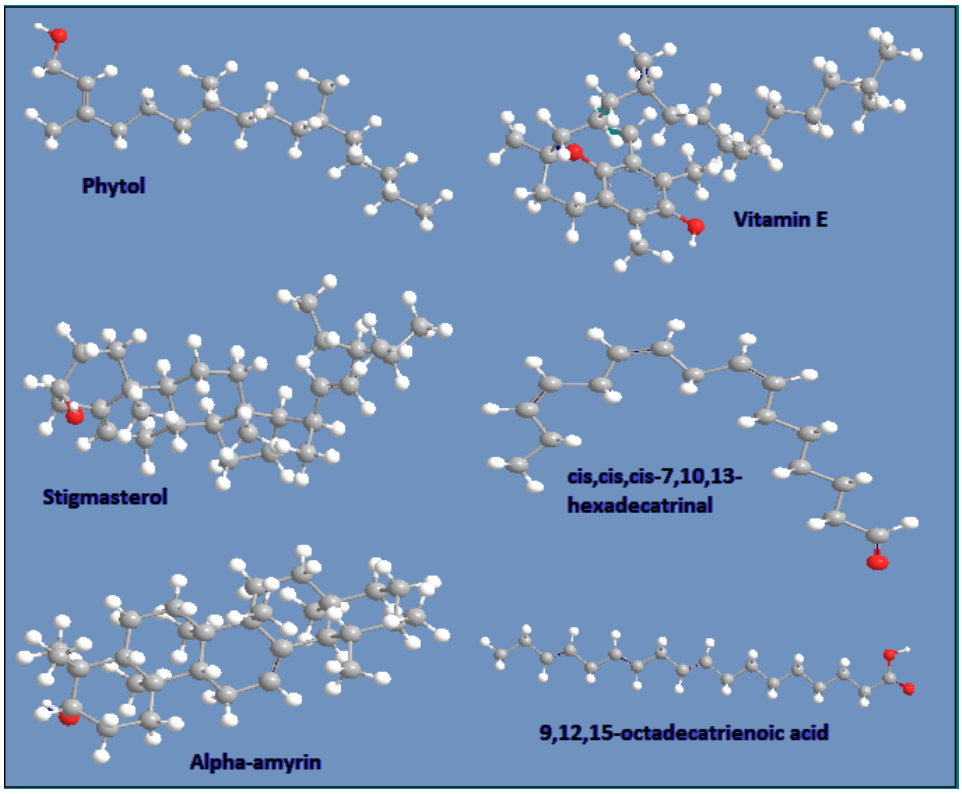


Figure 2. Antioxidant chemical compounds from the ethanol extract of *C.odorata.*

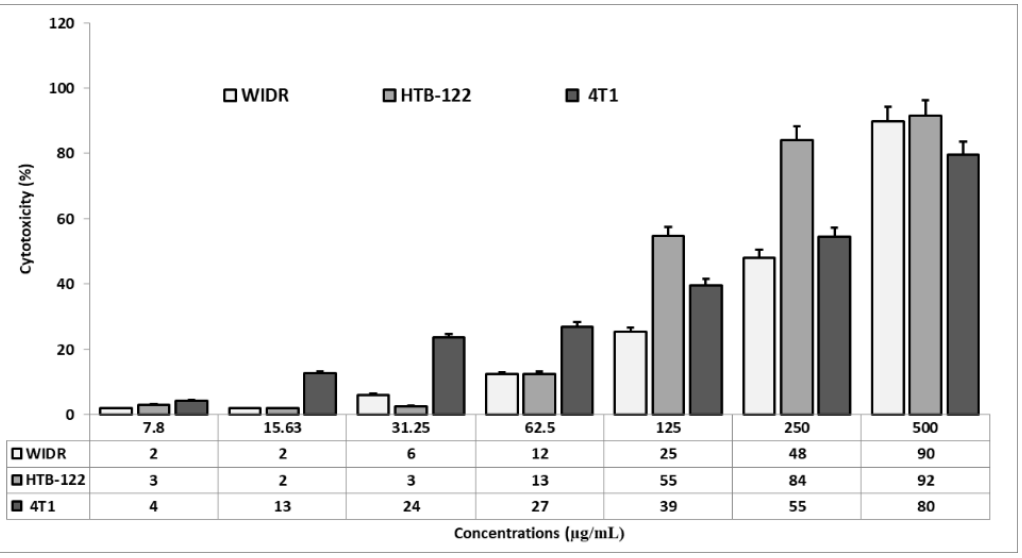
Table 1. Gas Chromatography-Mass Spectrometry evaluation of chemical compound of *C. odorata* ethanol extract

No.	Chemical compound	Retention time (min)	Quality (%)	Contents (%)
1	Caryophyllene	22.935	51	1.18
2	Hexadecanoic acid, methyl ester	27.389	99	2.11
3	Hexadecanoic acid	28.244	99	4.95
4	Methyl linolenate	28.693	99	3.49
5	Phytol	28.831	91	10.46
6	9,12,15-Octadecatrienoic acid	29.341	99	12.81
7	10,12-Hexadecadien-1-o1	29.927	78	2.93
8	(+) - Longifolene	30.168	70	2.61
9	Hexadecanoic acid, 2,3- dihydroxy propyl ester	30.885	60	13.67
10	Cyclotetracosane	31.465	99	1.26
11	cis,cis,cis-7,10,13-Hexadecatrienal	31.747	86	7.36
12	Squalene	32.009	95	3.43
13	(+) - (P,IR,3S)-Dimethoxy-2-mehyl-1-napththyl) -6,8-dimethoxy-1,1,3-trimethyl-1,2,3,4-tetrahydroisoquinoline (+)-O-methylancistrolcline	32.264	83	1.34
14	(+) - (P,IR,3S)-5-(4,5-Dimethoxy-2-mehyl-1-napththyl) -6,8-dimethoxy-1,1,3-trimethyl-1,2,3,4-tetrahydroisoquinoline (+) -O-methylancistrolcline	32.389	83	3.69
15	5-Hydroxy-2-(4-hydroxyphenyl)-7-methoxy-2,3-dihydro-4H-chromen-4-one	33.133	74	2.86
16	2 (3H)-Phenanthrenone, 4,4A-dihydro-4A-methyl-	33.602	58	3.8
17	1,4-Napthalenedione, 2,2'-(3-methylbutylidene) bis 3-hydroxy-	33.802	90	2.14
18	Vitamin E	34.478	98	2.03
19	Stigmasterol	36.091	99	3.94
20	Beta-sitosterol	36.877	97	3.03
21	Olean-12-En-3-ol	37.346	94	1.53
22	Alpha-amyrin	38.112	86	4.29

Table 2. Property engine and bioactivity score of the chemical compound of *C. odorata.*

Chemical compound	Property engine					Bioactivity score					
	miLogP	TPSA	MW	NVT	NROTB	GPCR ligand	ICM	KI	NRL	PI	EI
Phytol	6.76	20.23	296.54	1	13	0.11	0.16	- 0.32	0.35	0.00	0.31
cis,cis,cis-7,10,13-hexadecatrienal	5.4	17.07	234.38	1	11	0.17	0.46	- 0.34	0.05	0,07	0.45
9,12,15-octadecatrienoic acid	5.84	37.3	278.44	1	13	0.33	0.23	- 0.19	0.35	0.13	0.42
Vitamin E	9.04	29.46	430.72	1	12	0.25	0.14	- 0.21	0.41	0.28	0.24
Stigmasterol	7.87	20.23	412.7	1	5	0.12	- 0.08	- 0.48	0.74	- 0.02	0.53
Alpha-amyrin	8.08	20.23	426.73	1	0	0.22	- 0.02	- 0.41	0.79	0.19	0.60

TPSA: Topological polar surface area; NVT: Nviolations; MW: Molecule weight; NROTB: Number of the rotatable bond; GPCR: G-protein coupled receptors; ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand; PI: Protease inhibitor; EI: Enzyme inhibitor.



	7.8	15.63	31.25	62.5	125	250	500
WIDR	2	2	6	12	25	48	90
HTB-122	3	2	3	13	55	84	92
4T1	4	13	24	27	39	55	80

Figure 3. The cytotoxicity effect of *C. odorata* on the WiDr, HTB, and 4T1 cancer cells.

C. odorata leaves ethanolic extract (125-500 µg/mL) showed higher cytotoxicity against cancer cells WiDr, HTB, and 4T1 than the lowest concentrations (7.8-62.5 µg/mL).

The impact can cause the supply of nutrients and oxygen to cells to stop. Even these cells cannot protect themselves so that several active components of the test material will concentrate on cells, and cells become toxic. This phenomenon can explain that several active compounds in the extract ingredients disrupt these cells' function caused reactive oxygen species (ROS) and cell respiration system is to become unstable (Phaniendra et al., 2015). It is expected to occur when some natural substances, including *C. odorata*, interact with cancer cells. The *C. odorata* has anti-bacterial, inflammatory properties with relatively high antioxidants to prevent cancer cells' development (Kusuma et al., 2014). These polyphenols in *C. odorata* could maintain healthy cells' integrity and are toxic to bacterial and cancer cells. It hopes to protect the tissue cells and reduce the cancer cells' virulence. More than the effect increased the apoptosis and cytotoxicity (Thang et al., 2001).

The research results reported that *C. odorata* had a high quantity of total polyphenols and flavonoids. Also, *C. odorata* has strong antioxidant power. This antioxidant capacity has played a role in eliminating free radicals produced by cancer cells (Shrivastava et al., 2019). This property is in line with the cytotoxicity capability of *C odorata* to the WiDr, HTB, and 4T1 cancer cells (Fig. 3). The antioxidants have of *C. odorata* may interfere with cancer cells' development by inhibiting cancer cell kinase enzymes. Kinase enzymes as proteins' role in catalyzing protein phosphorylation regulate various cellular functions, such as proliferation, cell cycle, apoptosis, motility, growth, and differentiation, for cancer cells' survival and spread (Cicenas et al., 2018). Table 2 shows that *C. odorata* is negative to a kinase inhibitor, so its action requires ATP co-factors so that the kinase inhibitor can play an active role (Glickman, 2012). The cytotoxicity activity of *C. odorata* against cancer cells can be made possible by the high activity of the ion channel modulator of the six compounds found to be antioxidant (Table 1). Ion channel modulator works to interfere with cancer cells' modulation to develop by blocking and opening ions on the cell wall surface (Burke Jr and Bender, 2019). Apart from that, G protein-coupled receptors (GPCRs) for G protein-coupled inward rectifier potassium channels (GIRKs) and M channels. Ion channels are always modulated by reuptake inhibitors and releasing agents (Birch et al., 2004).

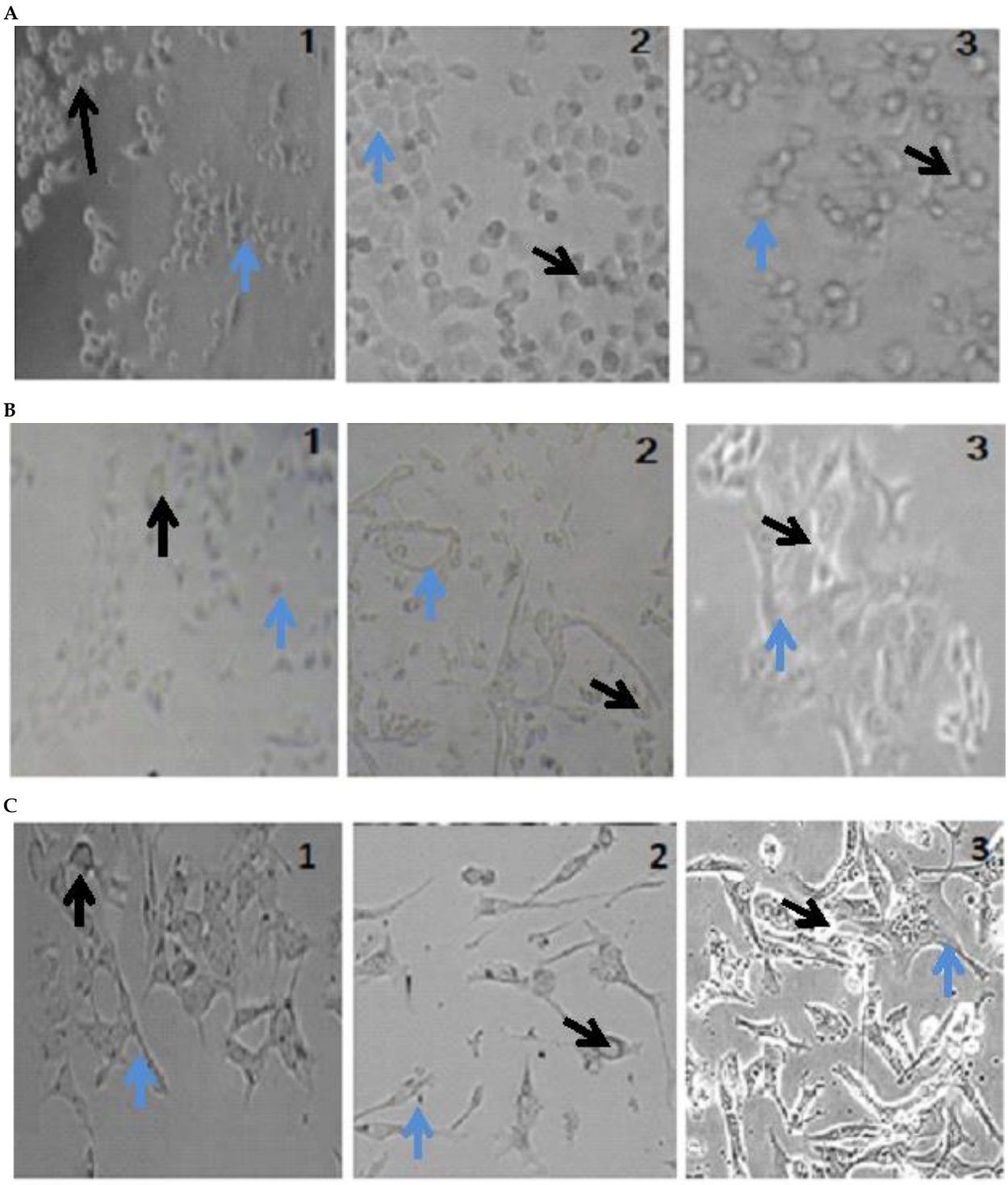


Figure 4. Profile of MTT assay of cancer cells.

(A) WiDr cells (A1. 250 µg/mL; A2. 500 µg/mL; A3. Doxorubicin 100 µg/mL). **(B)** 4T1. (B1. 250 µg/mL; B2. 500 µg/mL; B3. Doxorubicin 100 µg/mL). **(C)** HTB (C1. 250 µg/mL; C2. 500 µg/mL; C3. Doxorubicin 100 µg/mL). Blue arrow (normal cell) and black arrow (cytotoxicity). Magnification 400×

The ionic value was assayed to describe the pharmacodynamic and pharmacokinetic properties of *C. odorata*. The results show the properties of ionic dissolved oxygen, conductivity, and total dissolved solids, offering a balanced anti-activity percentage. It means that the reactive intensity always influences the action during the reuptake inhibitor channel on the cell surface. It has correlated reduce oxygen by increasing the area's hydrophobicity and cell surface during the test material response (Zhang et al., 2014). Furthermore, the conductivity possessed by *C. odorata* can disrupt the communication system between cells and intra-cell so that it can interfere with the protein synthesis process so that cancer cells do not develop. The total dissolved solids in question dissolve all the residues of cancer cell metabolism during reactions with the test material. This character controls and keeps healthy cells from becoming infected because the residue is harmful products (Valko et al., 2006). Besides, the ionic quality of *C. odorata* may increase enzyme inhibitor activity. This enzyme's increase can bind to other enzymes from cancer cells, such as catalase enzymes, to reduce cancer cells' virulence activity (Doskey et al., 2016).

Fig 2 shows the six compounds that act as antioxidants that have varied n-atoms, nON, nOHNH. The bioactivity score of six antioxidant compounds determines the degree of cytotoxicity (Table 2). These results illustrate that the role of each active compound can cause cells to become toxic. However, this study did not purify these compounds. It can confirm that they will be active during the cancer cells' response (Wang et al., 2012). Fig. 3 explains that the concentrations of 125, 250, and 500 µg/mL were determinant of the degree of toxicity of colorectal cancer cells (WiDr) and breast cancer cells (HTB and 4T1). It means that the higher the concentration of the element or active compound, the higher cytotoxicity. It ascertained that the concentration quantity is in line with the total amount of flavonoids and phenolics as antioxidants in *C. odorata*. The bioactivity score analysis results showed that all compounds suspected of being antioxidants had a nuclear receptor ligand. These receptors act as initiators of DNA transcription regulation to produce lipophilic proteins. The nuclear receptor ligand of *C. odorata* responds to cancer cells' lipophilic receptors (Weikum et al., 2018). It has shown *C. odorata* ability to inhibit the growth of colorectal and breast cancer cells (Fig. 4).

The cytotoxicity relationship of the two cancer cells by *C. odorata* could be possible that this natural material has protease inhibitors that can inhibit the development of infectious agents such as viruses as a result of the spread of cancer. This protein can prevent viral replication, which can exacerbate infection by inhibiting the proteolytic cleavage of protein precursors required by pathogens to produce disease (Patick and Potts, 1998). It means that these protease inhibitors can prevent secondary infection from breast and colorectal cancer. Besides, enzyme inhibitors that are owned by several *C. odorata* compounds can bind cancer cell metabolic enzymes to reduce nutrient intake and inhibit the formation of enzyme-substrate complexes' and prevent catalytic reactions so that they can kill pathogens and improve metabolic imbalances (Lupien et al., 2019). It assumed that *C. odorata* could balance average cell growth and decrease (apoptosis) against cancer cells (Robin et al., 2018).

CONCLUSIONS

The *C. odorata* has antioxidant capacity with a stable ionic value. The total flavonoid and phenolic quantities of *C. odorata* can increase the level of cytotoxicity to WIDR, HTB, and 4T1 cancer cells. The highest concentrations of *C. odorata* have significant cytotoxicity on the third cancer cells.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

ACKNOWLEDGMENTS

We acknowledge Syiah Kuala University by Professor Research Project Grant, No. 272/UN11/SPK/PNBP/2020.

REFERENCES

Abdulkareem IH (2013) Aetio-pathogenesis of breast cancer. Niger Med J 54: 371–375.

Adedapo AA, Oyagbemi AA, Fagbohun OA, Omobowale TO, Yakubu MA (2016) Evaluation of the anticancer properties of the methanol leaf extract of *Chromolaena odorata* on HT-29 cell line. J Pharmacog Phytochem 5: 52–57.

Alberga D, Trisciuzzi D, Montaruli M, Leonetti F, Mangiatordi GF, Nicolotti O (2018) A new approach for drug target and bioactivity prediction: The multi fingerprint similarity search algorithm (mussel). J Chem Inf Model 59: 586–596.

Armstead MY, Bitzer-Creathers L, Wilson M (2016) The effects of elevated specific conductivity on the chronic toxicity of mining influenced streams using *Ceriodaphnia dubia*. PlosOne 11: e0165683.

Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A (2018) How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? Cell Death Differ 25: 104–113.

Balaban S, Shearer RF, Lee LS, van Geldermalsen M, Schreuder M, Shtein HC, Grewal T (2017) Adipocyte lipolysis links obesity to breast cancer growth: adipocyte-derived fatty acids drive breast cancer cell proliferation and migration. Cancer Metab 5: 1.

Birch PJ, Dekker LV, James IF, Southan A, Cronk D (2004) Strategies to identify ion channel modulators: current and novel approaches to target neuropathic pain. Drug Discov Today 9: 410–418.

Burke Jr KJ, Bender KJ (2019) Modulation of ion channels in the axon: mechanisms and function. Front Cell Neurosci 13: 221.

Campillo N, Falcones B, Otero J, Colina R, Gozal D, Navajas D, Farré R, Almendros I (2019) Differential oxygenation in tumor microenvironment modulates macrophage and cancer cell crosstalk: novel experimental setting and proof of concept. Front Oncol 9: 43.

Cicenas J, Zalyte E, Bairoch A, Gaudet P (2018) Kinases and cancer. Cancers 10: 63

Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15(10): 7313–7352.

Doskey CM, Buranasudja V, Wagner BA, Wilkes JG, Du J, Cullen JJ, Buettner GR (2016) Tumor cells have decreased ability to metabolize H_2O_2: Implications for pharmacological ascorbate in cancer therapy. Redox Biol 10: 274–284.

Fattahi S, Zabihi E, Abedian Z, Pourbagher R, Ardekani AM, Mostafazadeh A, Akhavan-Niaki H (2014) Total phenolic and flavonoid contents of aqueous extract of stinging nettle and in vitro antiproliferative effect on hela and BT-474 Cell lines. Int J Mol Cell Med 3: 102–107.

Glickman JF (2012) Assay Development for Protein Kinase Enzymes In: Markossian S, Sittampalam GS, Grossman A, Brimacombe K, Arkin M, Auld D, Austin CP, Baell J, Caaveiro JMM, Chung TDY, Coussens NP, Dahlin JL, Devanaryan V, Foley TL, Glicksman M, Hall MD, Haas JV, Hoare SRJ, Inglese J, Iversen PW, Kahl SD, Kales SC, Kirshner S, Lal-Nag M, Li Z, McGee J, McManus O, Riss T, Saradjian P, Trask OJ Jr, Weidner JR, Wildey MJ, Xia M, Xu X, editors. Assay Guidance Manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences, pp. 2004.

Kamarlis RK, Lubis MND, Hernowo BS, Kar AS, Gani BA (2017) Frequency and distribution of cytoplasmic and nuclear P63 in recognizing the basal-like and non-basal-like types of breast cancer based on age. Asian. J Pharm Clin Res 11: 195–199.

Khan T, Dixit S, Ahmad R, Raza S, Azad I, Joshi S, Khan AR (2017) Molecular docking, PASS analysis, bioactivity score prediction, synthesis, characterization and biological activity evaluation of a functionalized 2-butanone thiosemicarbazone ligand and its complexes. J Chem Biol 10: 91–104.

Kouamé PB, Jacques C, Bedi G, Silvestre V, Loquet D, Barillé-Nion S, Tea I (2013) Phytochemicals isolated from leaves of *Chromolaena odorata*: impact on viability and clonogenicity of cancer cell lines. Phytother Res 27: 835–840.

Kusuma IW, Arung ET, Kim YU (2014) Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. Food Sci Hum Well 3(3-4): 191–196.

Lupien LE, Dunkley EM, Maloy MJ, Lehner IB, Foisey MG, Ouellette ME, Lewis LD, Pooler DB, Kinlaw WB, Baures PW (2019) An inhibitor of fatty acid synthase thioesterase domain with improved cytotoxicity against breast cancer cells and stability in plasma. J Pharmacol Exp Ther 371(1): 171–185.

Maulida PA, Putri DA, Fatmawati S (2019) Free radical scavenging activity of *Chromolaena odorata* L. leaves. IPTEK J Technol Sci 30(3): 73–75.

Patick A, Potts K (1998) Protease inhibitors as antiviral agents. Clin Microbiol Rev 11(4): 614–627.

Phaniendra A, Jestadi DB, Periyasamy L (2015) Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 30: 11-26.

Rajamanickam S, Agarwal R (2008) Natural products and colon cancer: Current status and future prospects. Drug Develop Res 69: 460–471.

Ranke J, Cox M, Müller A, Schmidt C, Beyersmann D (2006) Sorption cellular distribution, and cytotoxicity of imidazolium ionic liquids in mammalian cells–influence of lipophilicity. Toxicol Environ Chem 88(2): 273–285.

Robin T, Reuveni S, Urbakh M (2018) Single-molecule theory of enzymatic inhibition. Nat Commun 9: 779.

Rosenbaum, DM, Rasmussen SGF, Kobilka BK (2009) The structure and function of G-protein-coupled receptors. Nature 459: 356–363.

Salazar-Aranda R, Pérez-Lopez LA, Lopez-Arroyo J, Alanís-Garza, BA, Waksman de Torres N (2011) Antimicrobial and antioxidant activities of plants from northeast of

Mexico. Evid Based Complement Alternat Med 2011: 536139.

Santos-Sánchez NF, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B (2019) Antioxidant compounds and their antioxidant mechanism. IntechOpen. 1–28. DOI: 10.5772/intechopen.85270.

Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L (2011) Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med 8(1): 1–10.

Savjani KT, Gajjar AK, Savjani JK (2012) Drug solubility: importance and enhancement techniques. ISRN Pharm 2012: 195727.

Shrivastava A, Aggarwal LM, Mishra SP, Khanna HD, Shahi UP, Pradhan S (2019) Free radicals and antioxidants in normal versus cancerous cells—An overview. Indian J Biochem Bio 56(1): 7–19.

Siddiqui N, Rauf A, Latif A, Mahmood Z (2017) Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata* Benth). J Taibah Univ Med Sci 12: 360–363.

Sirinthipaporn A, Jiraungkoorskul W (2017) Wound healing property review of Siam weed, *Chromolaena odorata*. Pharmacog Rev 11: 35–38.

Thang PT Teik LS, Yung CS (2001) Antioxidant effects of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine–xanthine oxidase induced damage. Burns 27: 319–327.

Valko M, Rhodes C, Moncol J, Izakovic MM, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1): 1–40.

Wang H, Oo Khor T, Shu L, Su ZY, Fuentes F, Lee JH, Tony Kong AN (2012) Plants vs. cancer: a review on natural phytochemicals in preventing and treating cancers and their druggability. Anticancer Agents Med Chem 12(10): 1281–1305.

Weikum ER, Liu X, Ortlund EA (2018) The nuclear receptor superfamily: A structural perspective. Protein Sci 27: 1876–1892.

WHO (2018) Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer [Consulted September 12, 2018]

Zhang R, Li X, Shi Y, Shao Y, Sun K, Wang A, Sun F, Liu W, Wang D, Jin J, Li Y (2014) The effects of LPM570065, a novel triple reuptake inhibitor, on extracellular serotonin, dopamine and norepinephrine levels in rats. PlosOne 9(3): e91775.

AUTHOR CONTRIBUTION:

Contribution	Yusuf H	Husna F	Gani BA
Concepts or ideas	x		x
Design	x		x
Definition of intellectual content		x	
Literature search		x	
Experimental studies	x		x
Data acquisition	x		
Data analysis	x		x
Statistical analysis			x
Manuscript preparation	x		x
Manuscript editing	x		x
Manuscript review	x	x	x

Citation Format: Yusuf H, Husna F, Gani BA (2021) The chemical composition of the ethanolic extract from *Chromolaena odorata* leaves correlates with the cytotoxicity exhibited against colorectal and breast cancer cell lines. J Pharm Pharmacogn Res 9(3): 344–356.