

Phytochemical analysis and cytotoxic activities of two distinct cultivars of *Ganyong* rhizomes (*Canna indica*) against the WiDr colon cancer cell line

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Abstract. Ifandari, Widyarini S, Nugroho LH, Pratiwi R. 2020. *Phytochemical analysis and cytotoxic activities of two distinct cultivars of Ganyong rhizomes (Canna indica) against the WiDr colon cancer cell line. Biodiversitas 21: 1660-1669.* *Canna indica* L is an edible plant with high nutrient content and positive health benefits. In Indonesia, edible canna is divided into two cultivar groups: red edible canna and green edible canna. *Canna indica* L. is investigated in this study as a potential medicinal plant for colon cancer in WiDr cell lines. The phytochemical contents of two distinct cultivars of edible canna (known in Indonesia as *ganyong*), namely red edible canna and green edible canna. These two discrete samples were extracted by using two different solvents, dichloromethane and ethanol, followed by evaluation using Fourier-transform infrared (FTIR) spectroscopy in order to detected functional group. In the analysis of the composition of the elements of chemical compounds, the samples subsequently injected into the liquid chromatography–high-resolution mass spectrometry (LC-HRMS). The cell viability assay was performed to evaluate the cytotoxic activities of extracted compounds from two cultivars of edible canna in two different solvents. FTIR results demonstrated that the red and green edible canna extracts contained 17 and 18 functional group compounds, respectively. Furthermore, the cell viability was lower in WiDr cells treated with dichloromethane extract of red than green edible canna or the ethanol extract of red edible canna. Notably, LC-HRMS results of the most potent dichloromethane extract of red edible canna revealed the presence of fatty acid compounds. These findings indicate that the two cultivars of edible canna could potentially be utilized as promising candidates for colon cancer treatment.

Keywords: *Canna indica*, cytotoxic activity, phytochemical compound

INTRODUCTION

Canna indica L. is known locally as *ganyong* and has been widely recognized as an edible plant since ancient times. Its rhizome, which is tuberous and starchy, can be boiled and eaten (Vankar and Srivastava 2018). Edible canna is consumed by removing the starchy component (Carolina and Ilmi 2016), and it has also been used as complementary starch in the food and beverage home industry (Hasanah and Hasrini 2018; Vankar and Srivastava 2018). Despite the edible nature of canna rhizomes or bulbs, consumption of edible canna in Indonesia is limited due to lack of awareness regarding its nutritional content and processing. Edible canna, mostly found in Indonesia, comprises two cultivars, namely red and green edible canna. The difference between these two types is based on morphological characters. In general, the distinction has to do with tuber color, where red edible canna has a visibly reddish color to the scales and shoots, while green edible canna has a white rhizome, brown tuber scales, and green shoots (Sari et al. 2016). Most studies on edible canna are limited to morphological differences and nutritional products. However, little is known about the differences in chemical composition between the two

cultivars as well as the cytotoxic activities of edible canna against colon cancer.

It has been reported that edible canna contains nutrients such as carbohydrates, proteins, lipids, fiber, ash or inorganic materials (Okonwu and Ariaga 2016), calcium, phosphorus, iron, and vitamin C (Pudjihastuti et al. 2018). Moreover, edible canna or *ganyong* tuber contains another macro compound, specifically butyric acid (Lim 2016). Furthermore, it contains several secondary metabolite compounds such as alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, saponin, phlobatinin, betulinic acid, oleanolic acid, lignin, furfurals, and hemicellulose (Lamaeswari and Anathi 2012; Al-Snafi 2015).

Previous studies showed that the extract of edible canna root and rhizomes exhibited antinociceptive, anthelmintic (Nirmal et al. 2007), antimicrobial (George 2014), antidiabetic, and antilipidemic activities (Subhash et al. 2017). Edible canna demonstrated its ability to protect against oxidative-stress-induced ischemia in the rat (Talluri et al. 2017). The anticancer activity of edible canna was studied in previous research. Burhannudin et al. (2018) demonstrated that edible canna starch decreases the expression of adenomatous polyposis coli and inducible nitric oxide synthase in rats induced by AOM/DSS.

The cytotoxic activity of natural substances against cultured cancer cells is used as a basis for screening candidate anticancer agents. Cytotoxicity is based on the death of cancer cells due to treatment and can be detected by using the MTT test (Bahuguna et al. 2017). MTT assays are used in cytotoxic tests of various cancer cell lines, such as MCF-7, MDA-MB468, HepG2, Hela, Vero, HS5.T, HS39T, MCF-7, and HT29, SW872, SW982, and Caco-2 (Demirgan et al. 2016; Yumrutas et al. 2015; Antoney et al. 2016; Lombardi et al. 2017; Alam et al. 2017).

Fourier transform infrared (FT-IR) spectroscopy is a method of detecting chemical bonds by using an infrared spectrum that is absorbed by the material. This method shows the intensity of the peak absorbance of the functional groups (Munajad et al. 2018). FTIR method can be used in both qualitative and quantitative analysis, and detection includes molecular conformation and interaction between neighboring molecules. The advantages of this method are fast, accurate, simple, and nondestructive (Amir et al. 2013).

Liquid chromatography, coupled with high-resolution mass spectrometry (LC-HRMS), has considered as the new gold standard in LC-MS analysis. This method can carry out quantitative, qualitative, quan/qual, and omics analyses (Rochat 2018). The ability of LCHRMS can be used in capturing targeted/untargeted analysis and provide more comprehensive information about exact molecular mass, elemental composition, and detailed molecular structure (Andra et al. 2017; Campmajo et al. 2019). LC-HRMS is a selective and sensitive full-scan mode. This method can analyze all ions in a large mass range (Rochat 2018). The process of identifying chemical structures in LCHRMS is carried out by analyzing ion products from an abundance of ion fragments (Yu et al. 2014).

Therefore, this study is conducted to investigate various phytochemical compounds of red and green cultivars of edible canna against WiDr colon cancer cell line. Based on the polarity of the phytochemical compound, dichloromethane and ethanol were used to extract the red and green edible canna.

MATERIALS AND METHODS

Plant material and preparation

Edible canna rhizomes of the green and red cultivars were purchased from Mojosongo, Boyolali, Central Java, Indonesia, at an approximate age of 8 months. The identification process was carried out according to a previous study by Sari et al. (2016). Briefly, all parts of the edible green and red canna plant were characterized. The flowers, fruit, leaves, rhizomes, and shoots of the plant were observed. Plant determination was conducted manually by a specialist from the Plant Systematics Laboratory, Faculty of Biology Universitas Gadjah Mada. Subsequently, a specimen (No. 050/2016/FFUSB) was deposited in the herbarium of the Laboratory of Phytochemical, Faculty of Pharmacy, Universitas Setia Budi, Surakarta, Indonesia.

Procedures

Extraction of edible rhizome from Canna indica

Green and red edible canna rhizomes were weighed to 10 kg, washed with water, and left to dry at room temperature. Fresh and clean rhizomes were sliced thinly and dried in an oven at 45 °C for seven days. Subsequently, dried powder of rhizome was prepared using a grinding machine (Maksindo, Indonesia). The powder was sifted in 40-60 mesh, kept in a bottle, and refrigerated. Plant extraction was performed separately using multilevel maceration methods with dichloromethane and ethanol (Merck, Germany), according to Sruthi and Indira (2016). Successive maceration was performed to separate the secondary metabolite compounds contained in a mixture of non-polar and polar samples.

Powder of edible canna rhizomes (250 g) was poured into a dark bottle, added to 2500 ml dichloromethane, closed tightly, kept for 72 hours and shaken three times a day. After 72 hours, the extract was filtered using Whatman paper (Sigma, USA) and then macerated twice with dichloromethane. The filtrates were evaporated with a rotary evaporator (IKA, Malaysia) at 40 °C and 150 rpm. The residue after completion of maceration in dichloromethane was dried and then macerated three times using ethanol as the solvent. The ethanol maceration process was the same as dichloromethane maceration processing. The obtained extract was weighed and stored in a glass container at 4 °C.

FTIR tape pattern inspection

The extracts were made into pellets using KBr for FTIR analysis, and the thin film was prepared by applying pressure. The samples were loaded into an FTIR spectroscope, and the spectroscopy results were recorded on a Shimadzu FTIR Spectrometer 8201 Series, in the scan range between 4,000 and 400 cm^{-1} (Florence and Jeeva 2015). Data absorbance was then analyzed by comparison with standards from table chem.libretexts.org to predicted chemical bonds and functional groups. The spectral peaks were visualized graphically.

Preparation of cell lines

The WiDr human colon carcinoma cell line was obtained from the Parasitology Laboratory of the Faculty of Medicine, Universitas Gadjah Mada. WiDr cells were prepared from stock cultures and grown in tissue culture petri dishes. The complete culture medium consisted of RPMI (Gibco, USA), fetal bovine serum (FBS) (Gibco, USA), antibiotics (penicillin-streptomycin), and Fungizone (Gibco, USA). A 20 ml aliquot of FBS 10% was added to a sterile bottle, followed by 2 ml of penicillin and streptomycin mixture (1%) and 1 ml fungizone (0.5%). The mixture was added to the bottle containing 200 ml RPMI 1640 medium. Cells were grown in RPMI complete medium and cultured in a humidified incubator (Heraeus) with an air mixture containing 5% (v/v) CO_2 at 37 °C. Cells were passaged when the culture was 70%–80% confluent. Cells were harvested from the culture dish with trypsin-EDTA 0.025% (Gibco, Canada). RPMI medium was added to the petri dish to a volume of 5 ml and the cells

resuspended. A hemacytometer counting chamber was used to count and calculate the number of cells, and then they were prepared for seeding in 96-well plates at approximately $1 \times 10^4/100 \mu\text{l}$ (Meiyanto et al. 2017).

Cytotoxicity evaluation by MTT assay

Cytotoxic activities were evaluated by the MTT assay procedure based on the method of Meiyanto et al. (2011). Briefly, cells were grown in complete RPMI medium in 96-well plates (Iwaki, Japan) for 24 hours. Each extract was prepared as follows: 10 mg extract was added to 100 μl DMSO (99.5% pro GC, Sigma Aldrich, Germany) to prepare a stock solution with a concentration of 100,000 $\mu\text{g}\cdot\text{mL}^{-1}$ (ppm). The stock solution was diluted with complete RPMI medium to concentrations of 2000; 1500; 1000; 750; 500; 250; and 125 ppm for use in treatments. Each concentration was tested in triplicate. The cell medium in the plate was removed and 100 μl new medium with different concentrations of extracts was added to each well and incubated for 24 hours. Cell survival was determined with the MTT assay test. The cell medium was removed, and MTT reagent (Sigma, USA) that had been dissolved in 1 ml of PBS to 9 ml RPMI medium was added, to a volume of 100 μl /well. The plate was incubated in the dark for 3–4 hours. At the end of the treatment, 100 μl of stop solution consisting of 10% SDS (Merck, Germany) in HCN (Merck, Germany) 0.1 N was added to each well. The plate was then incubated overnight in the dark at room temperature. Absorbance readings were taken in an ELISA reader (Bio-Rad, USA) at 595 nm. The absorbance of each well was converted to the percentage of viable cells as follows:

$$\% \text{ Viable cells} = \frac{\text{Treated cell abs} - \text{Medium control abs}}{\text{Cell control abs} - \text{Medium control abs}} \times 100\%$$

Profiling the chemical compounds in the potential extract by liquid chromatography high-resolution mass spectrometry (LC-HRMS)

An LC-HRMS instrument with a Q Exactive Mass Spectrometer (Thermo Scientific, USA) was used to analyze the chemical compounds in the potential extract. The separation column used was the Hypersil Gold aQ column, 50 mm \times 1 mm, with 1.9- μm particle size. The oven temperature was set at 30 $^{\circ}\text{C}$. After pretreatment,

samples were diluted frequently in methanol before injection into the LC system. The A solvent contained a mixture of formic acid (0.1%) in water, and the B solvent contained a mixture of formic acid (0.1%) in acetonitrile. The flow rate was 40 $\mu\text{L}/\text{min}$. The total run time was 70 min. The Orbitrap (ESI) electrospray positive and negative ionization ion source was employed. The resolution was 17.500 to 70.000. One hundred milligrams of extract were dissolved in 500 μl methanol and homogenized. The suspension was filtered with a syringe filter of 0.45 μm . About 10 μl suspensions were injected into the LCHRMS and run for 70 minutes. The HRMS data were filtered with Compound Discoverer software to identify components related to the known compounds.

Data analysis

Statistical analyses were performed using SPSS version 21.0. The percentages of viable cells in each extract were analyzed with probit tests to define the inhibition concentration 50% values (IC_{50}). Data were expressed as mean \pm S.E.M. (Standard Error of the Mean).

RESULT AND DISCUSSION

Morphological variation in red and green edible canna (*Canna indica* L) plants

Rhizomes of edible canna that were used in this study were obtained from one location at Mojosongo Boyolali. This plant is commonly found in the region due to the customary consumption of edible canna by local people. According to the complete determination, edible canna was identified as *Plantae* kingdom, *Tracheophyta* division, *Liliopsida* class, *Zingiberales* order, *Cannaceae* family, *Canna* genus, and *Canna indica* L Species. From the species level, members of this group can be divided into two cultivars, namely the red and green cultivars. The distinct character of the two canna groups is known via morphological characteristics. Differentiation of two cultivars can be seen in every part of a plant, such as the flowers, sheaths, leaves, bud, rhizome, and fruit (Table 1). The red edible canna cultivar used in this study was identified in the indica variety group, whereas the green cultivar was identified in the flava variety group (Figure 1).

Table 1. Morphological characters of red and green edible canna cultivars

Parts of plant	Morphological character	
	Red edible canna	Green edible canna
Flower	Red Staminodia, red petala, red bracteal	Red Staminodia, white to yellowish petala, white bracteal
Sheaths color	Dark purple	Green
Leaf margin	Purple	Transparent
Bud and rhizome	Purple	Green strip purple
Fruit color	Green spot purple	Green

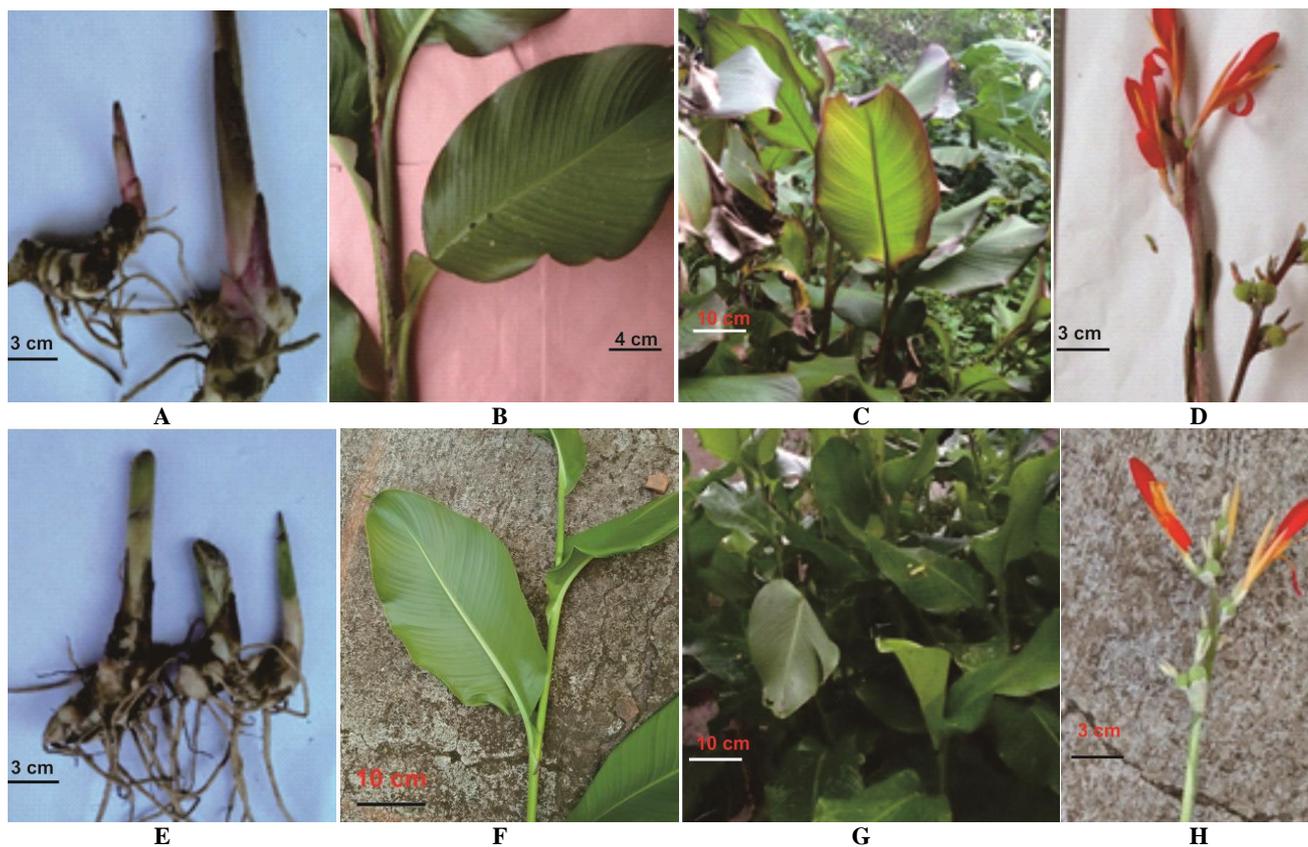


Figure 1. Morphological variation of red and green edible canna cultivars. Plant parts of the red canna cultivar are a. rhizome, b. stems, c. leaves, and d. flowers, respectively. While plant parts of the green canna cultivar are e. rhizome, f. stems, g. leaves, and h. flowers, respectively.

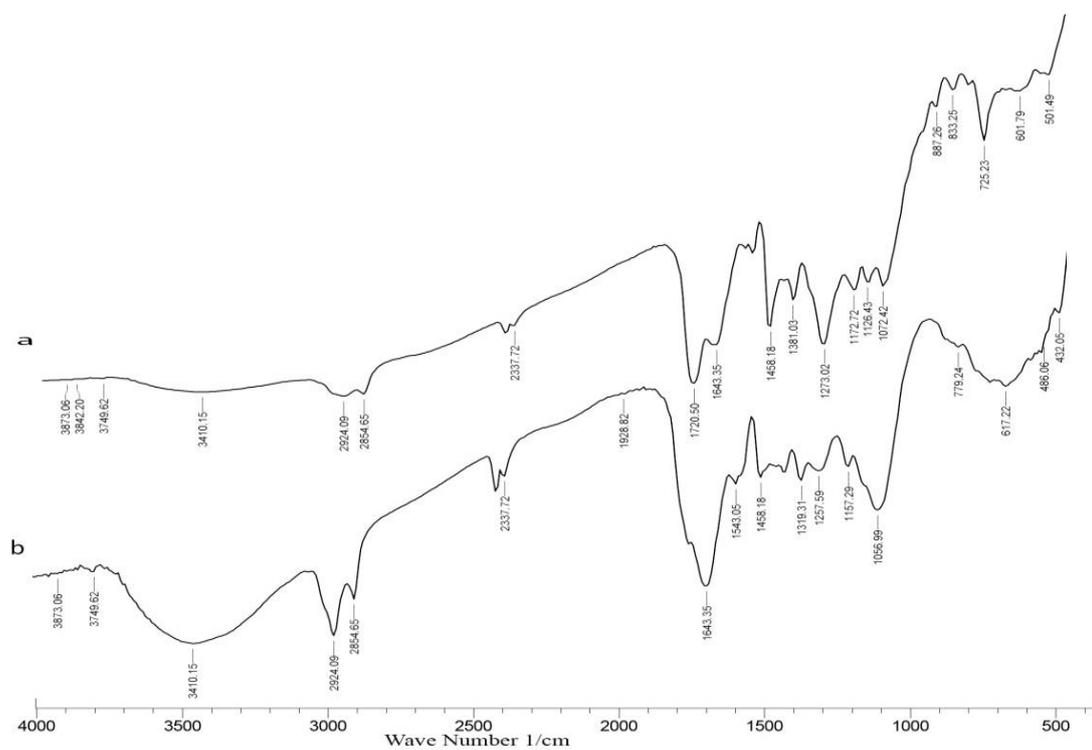


Figure 2. Spectra Pattern FTIR. (a) Dichloromethane extract of red edible canna (RD) and (b) dichloromethane extract of green edible canna (GD)

FTIR spectral pattern of red and green edible canna rhizome

The results of the study showed four spectral patterns of red edible canna dichloromethane extracts (RD), green edible canna dichloromethane extracts (GD), red edible canna ethanol extract (RE), and green edible canna ethanol extract (GE). FTIR spectra of red and green edible canna dichloromethane extracts showed different patterns. The absorbance bands between red and green edible canna dichloromethane extracts are slightly different in the region from 3700 to 3000, as well as from 1000 to 500 (Figure 2).

The major peaks were observed at 3410.15 and 617.22 in green edible canna dichloromethane extract. The differentiation of spectra bands was related to the chemical bonds of the functions of OH, NH, and halogen compounds. The difference spectra of red and green edible canna dichloromethane extract were not only from the patterns but also from the total peaks. The red edible canna dichloromethane extract has 20 peaks, while green edible canna has 18 peaks.

The spectra of red and green edible canna ethanol extracts showed similar shapes and patterns. The similarity of the spectral patterns of red and green edible canna ethanol extracts was found in the region of 3000 to 900 (Figure 3.). The major peaks were observed at 3456.44 in

red edible canna and 3371.57 in green edible canna ethanol extract. The difference in spectra between red and green edible canna ethanol extract was only in peak numbers. The red edible canna ethanol extract has 13 peaks, whereas green edible canna has 11 peaks.

The peak numbers were analyzed from the FTIR spectra to predict the chemical bonds and functional groups in each extract. The differences among the four types of edible canna extracts can be seen from a prediction of the chemical bonds it contains (Table 2).

From the FTIR spectra of four types of edible canna, 19 functional groups were predicted. The predicted functional groups were alcohols, amines, carbocyclic acids, alkanes, alkenes, anhydrous groups, esters, ketones, phenols, ethers, fluoro compounds, a sulfur compound, halogen compounds, benzene derivatives, nitro compounds, alkynes, isocyanates, allenes, and aromatic compounds. The functional groups that are owned by both types of red edible canna and green edible canna are alkanes, alkenes, ketones, phenols, a sulfur compound, and isocyanate. The functional groups that were only found in green edible canna were allenes, carbodiimide, azide, and aromatic compounds and those found only in the red canna were benzene derivatives.

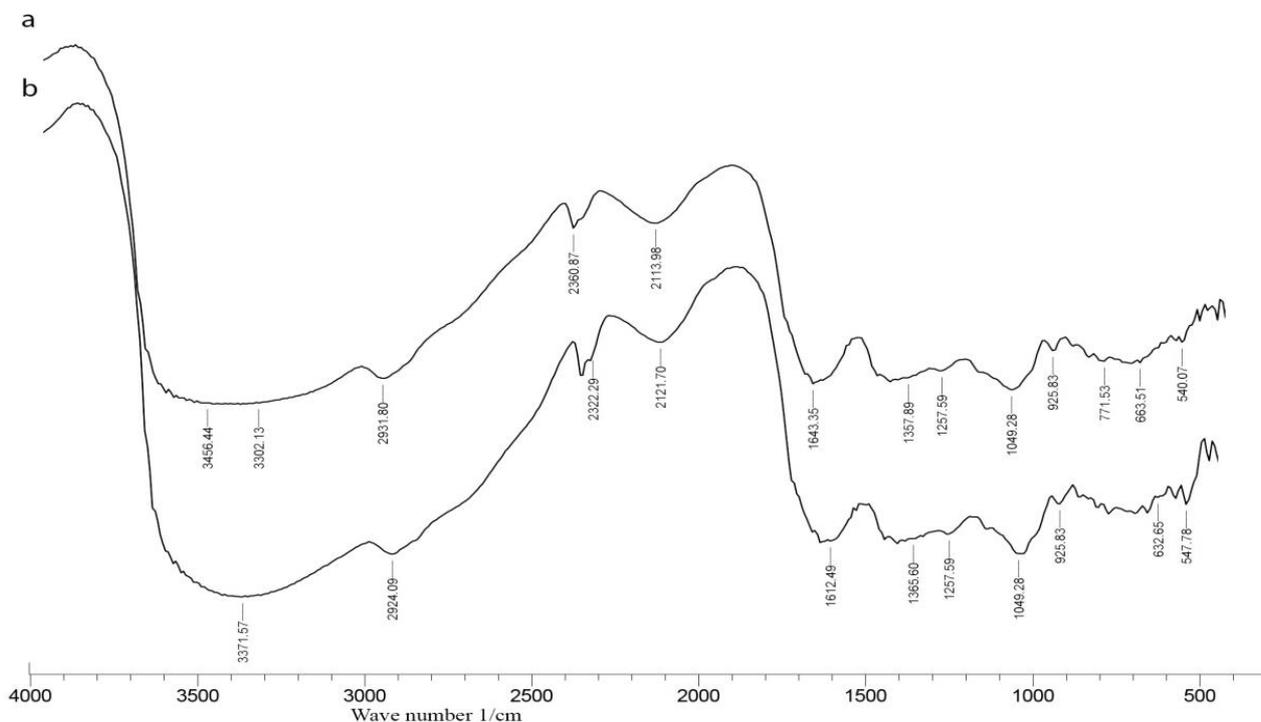


Figure 3. Spectra Pattern FTIR. (a) Ethanolic extract of red edible canna (RE) and (b) ethanolic extract of green edible canna (GE).

Table 2. FTIR spectral wavenumber values and functional groups obtained from the four types of edible canna extracts

Functional group	Red edible canna (<i>Canna indica</i> var. <i>indica</i>)		Green edible canna (<i>Canna indica</i> var. <i>flava</i>)	
	Dichloromethane (RD)	Ethanol (RE)	Dichloromethane (GD)	Ethanol (GE)
Alcohol				
O-H stretching	3410.15; 2924.09	3456.44; 3302.13; 2931.8	3410.15; 2924.09	3371.57; 2924.09;
O-H bending	1381.03	1357.89		1365.6;
C-O stretching	1172.72; 1126.43; 1072.42		1157.29; 1056.99	
Amine				
N-H stretching	3410.15; 2914.09; 2854.65	3456.44; 3302.13; 2931.8	3410.15; 2924.09; 2854.65	3371.57; 2924.09
N-H bending amine	1643.35	1643.35	1643.35	1612.49;
C-N stretching	1273.02; 1172.72; 1126.43; 1072.42	1049.28	1319.31; 1157.29; 1056.99	1049.28;
Carbocyclic acid				
O-H stretching	2924.09; 2854.65	2931.8	2924.09; 2854.65	2924.09;
C=O stretching	1720.5			
Alkane				
C-H stretching	2924.09; 2854.65	2931.8	2924.09; 2854.65	2924.09
C-H bending	1381.03			1365.6
Alkene				
C=C stretching		1643.35	1928.82; 1643.35	1612.49
C=C Bending	887.26; 833.25; 725.23		779.24	
Anhydrite				
C=O stretching	1720.5			
CO-O-CO		1049.28		1049.28
Ester				
C=O stretching	1720.5			
C-O stretching	1273.02; 1172.72	1257.59; 1049.28	1257.59	1257.59
Ketone				
C=O stretching	1720.5			1612.49
Phenol				
O-H bending	1381.03	1357.89	1319.31	1365.6
Ether				
C-O stretching	1273.02; 1126.43; 1072.42;	1257.59; 1049.28	1257.59; 1056.99	1257.59; 1049.28
Flouro compound				
C-F stretching	1381.03; 1273.02; 1172.72; 1126.43; 1072.42	1357.89; 1257.59; 1049.28	1319.31; 1257.59; 1157.29; 1056.99	1365.6; 1257.59
Sulfur compound				
S=O stretching	1381.03; 1172.72; 1126.43	1357.89; 1049.28	1319.31; 1157.29; 1056.99	1365.6; 1049.28
Halogen compound				
C-Cl stretching	833.25; 725.23; 601.79	771.53; 663.51	779.24; 617.22	632.65
C-Br stretching	601.79	663.51	617.22	632.65; 547.78
C-I stretching	501.49			547.78
Benzene derivative				
C-H bending	887.26; 833.25; 725.23	771.53		
Nitro compound				
N-O stretching		1357.89	1543.05	1365.6
Alkyne				
C≡C stretching		2113.98		2121.7
C-H stretching		3302.13		
Isocyanate				
N=C=S stretching		2113.98		2121.7
Allene				
C=C=C stretching			1928.82	
N=C=N stretching carbodiimide				2121.7
N=N=N stretching azide				2121.7
Aromatic compound				
C-H bending			1928.82	

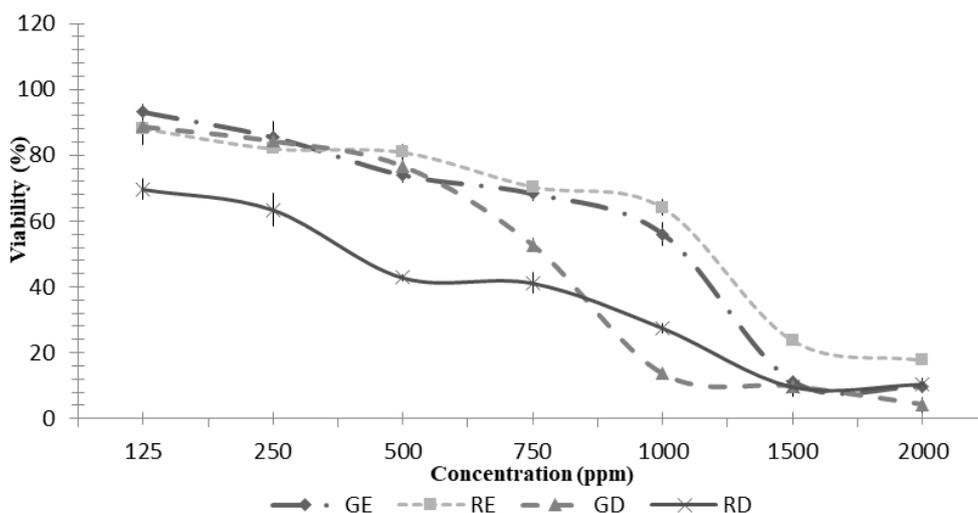


Figure 4. Percentage of viability colon cancer cells WiDr against the treatment of four types of extracts. Dichloromethane red canna extract (RD), ethanol red canna extract (RE), dichloromethane green canna extract (GD), and ethanol green canna extract (GE).

Viability of WiDr colon cancer cells

The cytotoxic activities of four types of extracts were observed from the percentages of living cells following treatment with each extract. A cytotoxic activity assay was tested in WiDr colon cancer cell lines to screen for anticancer activity of edible canna against colon cancer. The percentages of viable cells were decreased due to treatment with edible canna extracts in proportion to the increase in extract concentrations. The test concentrations used ranged from 125 ppm to 2000 ppm (Fig 4).

The highest percentages of cell death were found in the dichloromethane extract of red edible canna, followed by dichloromethane extract of rhizome green edible canna. Both types of extracts belong to semi-polar groups. Red edible canna extract and green edible canna extract with ethanol solvent exhibit lower toxicity compared to dichloromethane extract. The value of toxicity was assessed by 50% inhibition concentration (IC_{50}) value of each type of extract against the type of test cell. The IC_{50} value of dichloromethane extracts red edible canna was lower than ethanol extract. IC_{50} value of dichloromethane red edible canna extract was 361.83 ± 20.87 ppm and green edible canna extract was 561.57 ± 19.75 ppm against WiDr colon cancer cell lines. This value was lower than the IC_{50} value of ethanol extract of red edible canna at 979.57 ± 34.36 ppm and green edible canna extract at 809.90 ± 20.36 ppm. The IC_{50} values of four types of extracts (dichloromethane of red edible canna, dichloromethane of green edible canna, ethanol of red edible canna and ethanol of green edible canna) against

WiDr colon cancer cells were less than 1000 ppm. The most toxic extract against the WiDr cancer cell was red edible canna dichloromethane extract.

Chemical compound profiling in dichloromethane extract of red edible canna

Dichloromethane extract of red edible canna had the highest cytotoxic activity against WiDr colon cancer cells compared with other extract types. The phytochemicals analyzed by using the LCHRMS separation method. Spectra both positive and negative mode, with UV absorption maxima and retention time used as data. These data analyzed by Compound Discoverer software. Molecular ions are identified by software and matched with a database. Confirmed data are HRMS, MS / MS, retention time, and UV data. The final results of the LC-HRMS analysis of the dichloromethane extract of the red canna rhizome obtained the content of chemical compounds, and their percentage are presented in Table 3.

Most of the compounds contained in the extract belong to the group of esters and fatty acids. The compound bis (2-Ethylhexyl) phthalate was the largest component (51.269%) of the rhizome extract of red edible canna in the dichloromethane solvent. This compound belongs to the ester group. Existing compounds are further sequenced from the fatty acid group and are small in number a collection of compounds – compounds that provide high cytotoxic activity against WiDr colon cancer cells.

Table 3. Chemical compounds in dichloromethane red edible canna rhizome extract by LC-HRMS

Retention time	Compound	Formula	Mol wt	Relative percentage on the extracts (%)
0.977	Betaine	C ₅ H ₁₁ N O ₂	117.079	0.2359
1.026	Choline	C ₅ H ₁₃ N O	103.100	2.2513
5.485	4-Indolecarbaldehyde	C ₉ H ₇ N O	145.052	0.4471
7.617	Sedanolide	C ₁₂ H ₁₈ O ₂	194.130	0.2115
11.664	9S,13R-12-Oxophytodienoic acid	C ₁₈ H ₂₈ O ₃	292.202	0.5192
11.956	α -Eleostearic acid	C ₁₈ H ₃₀ O ₂	278.223	5.1784
12.231	(+)-ar-Turmerone	C ₁₅ H ₂₀ O	216.150	0.6758
12.279	N-Phenyl-1-naphthylamine	C ₁₆ H ₁₃ N	219.104	1.8988
12.594	9-Oxo-10 (E),12 (E)-octadecadienoic acid	C ₁₈ H ₃₀ O ₃	294.218	5.4003
14.881	1-Linoleoyl glycerol	C ₂₁ H ₃₈ O ₄	354.275	0.9390
15.031	α -Linolenic acid	C ₁₈ H ₃₀ O ₂	278.223	1.1313
16.136	Eicosapentaenoic acid	C ₂₀ H ₃₀ O ₂	302.223	1.5999
16.978	Oleamide	C ₁₈ H ₃₅ N O	281.270	0.4498
17.564	Docosatrienoic acid	C ₂₂ H ₃₈ O ₂	334.286	0.3068
17.626	Hexadecanamide	C ₁₆ H ₃₃ N O	255.255	0.4804
18.185	Bis (2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390.275	51.2691
19.061	Phthalic acid	C ₈ H ₆ O ₄	166.026	0.3174
19.904	Stearamide	C ₁₈ H ₃₇ N O	283.286	0.2371
20.059	11 (Z),14 (Z),17 (Z)-Eicosatrienoic acid	C ₂₀ H ₃₄ O ₂	306.254	0.7498
20.884	Erucamide	C ₂₂ H ₄₃ N O	337.333	0.6579

Discussion

In Indonesia, edible canna includes of two cultivar groups: the red and green cultivars. The basic grouping of the canna cultivars is based on the colors of the bud and leaf sheaths; pigmentation of the leaf margins and lower midrib surface; and color of the rachis, spatha, and fruit (Sari et al. 2016). The red edible canna was used in the research included in the *indica* variety group and green edible canna included in the *flava* variety group. These two varieties are widely cultivated in the Boyolali region. The *indica* variety of red edible canna is most commonly found in Indonesia, especially Java (Sari et al. 2016). The results of previous studies found that the diversity of red and green edible canna morphology did not depend on environmental factors in the area where plants grow. Genetic tracking supports this with the RAPD method, which shows that the genes cluster into two groups, as with the morphological characters, although the samples come from different regions (Sari et al. 2018). Morphological differences allowed differences in phytochemical contents. Morphology is a phenotypic character that can be formed from genotypic characters. Due to the differentiation of genetic factors between red and green edible canna, it is possible that the phytochemical contents of the two groups of edible canna are different.

Differences in the spectral patterns between the two cultivars of edible canna were found in the peak bands. The spectral patterns of the two varieties showed some differences in the dichloromethane solvent, while in ethanol, it did not. The peak bands in the dichloromethane solvent of red and green edible canna were more noticeable compared to ethanol extracts. This gives information that the difference in compounds of the two varieties lies in compounds that tend to be semi-polar to nonpolar. Phytochemical screening analysis of *Canna indica* rhizome

extracts found alkaloids, saponins, tannins, polyphenols, terpenoids, and flavonoids. Alkaloids are found in all types of extracts; the discovery knows this of O-H band bonds, N-H bands, C = O bonds, and C-H stretching groups. The four chemical bonds are generally found in the class of alkaloids (Sharmi 2016). The existence of terpenoids can be found in all four extracts. This is known from the presence of C-H stretch bond, O-H stretch, C = O stretch, C-O stretch Ester (Boughendjioua and Djeddi 2017; Mohandas and Kumaraswamy 2018). The lipid groups were known from C-H stretching vibration bound in region 3000 to 2800 cm⁻¹ (Starlin et al. 2012). Those chemical bonds were found in all extracts. The absence of a peak in the region of 2260 cm⁻¹ indicates edible canna rhizome both in red and green varieties do not contain cyanide groups, so this group is declared safe for foodstuffs (Singh and Mendhulkar 2015; Chunduri and Shah 2016). The difference in spectral patterns and groups of compounds possessed by red and green varieties was only slight in the functional groups of allenes, aromatic compounds, and aldehydes. A possible explanation is that these two varieties were grown in the same area with similar natural factors.

A cytotoxic activity test can be used as the basic principle for screening potential substances for suitability as anticancer agents. The MTT test was also used for screening anticancer activity of natural products such as *Cichorium intybus*, *Zataria multiflora*, *Morus alba*, *Eucalyptus camaldulensis* (Soltanian et al. 2017), *Cardiospermum halicacabum* L., *Gomphrena celosioides* Mart., and *Scoparia dulcis* L (Promraksa et al. 2019). The basic principle of the MTT assay test was based on the activity of mitochondria in living cells that form formazan crystalline. The dead cells did not form formazan crystalline. The dead cells and living cells are distinct and

give more precise results (Jardine et al. 2019). Cytotoxic activity of edible canna extract from two cultivars was tested in WiDr colon cancer cells.

Cell mortality is due to exposure to edible canna rhizome extract causing outside cell signal effects to induce cell death. The percentage of viable cells after exposure to four types of edible canna extract for 24 hours was different. The highest cytotoxic activity was seen in the dichloromethane red edible canna extract. The IC_{50} value of this extract was above 100 $\mu\text{g/ml}$, so it was included in the group with low anticancer activity. The second toxicity value was the dichloromethane extract of green edible canna rhizome. After that were the green and red edible canna ethanol extracts. The IC_{50} value of natural products possessed by edible canna rhizome is still below 1000 ppm, but above 100 $\mu\text{g/ml}$, so it is classified as having low anticancer activity (Soltanian et al. 2017). Although anticancer activity was low, this natural product can be used to preventative agents from cancer, especially colon cancer. This material was still in the form of crude extract so that further separation needed to obtain certain types of compounds that have high cytotoxic activity against colon cancer cells WiDr. Dichloromethane extract of red edible canna contained complex compounds.

The results of the analysis by LCHRMS showed several types of compounds that have cytotoxic activity, especially against cancer cells. The most abundant compound in red edible canna extract is bis (2-Ethylhexyl) phthalate, which belongs to the ester group. The type of compound isolated from plants and bacteria had a toxic activity to several types of cancer cells such as MCF-7, HEPG-2, HELA, and HCT-116 (Lotfy et al. 2018). The second group with a large percentage is a fatty acid group. The fatty acid groups consist of α -Eleostearic acid, α -Linolenic acid, 9-Oxo-10 (E), 12 (E)-octadecadienoic acid, Eicosapentaenoic acid, 9-Oxo-10 (E), 12 (E)-octadecadienoic acid and 1-Linoleoyl glycerol. The fatty acid group has the property of modulating the viability of cancer cells and modulating the ability of cell proliferation and death (Murray et al. 2015). Fatty acid groups such as Eicosapentaenoic acid and α -Linolenic acid could induced apoptotic activity and cell cycle in the WiDr cell line (Fauser et al. 2011). However, antiproliferative effects on HT-29 colon cancer cell lines have been reported by Karan and Erenler 2018. Amine fatty acid derivative groups such as Oleamide, Hexadecanamide, and Erucamide. Oleamide can increase cyclic AMP production by 50% in HeLa cells that express 5-HT₇ receptors (Ezzili et al. 2010). Betaine and choline are found in supplement form. The supplement will provide the effect of reducing the incidence of cancer (Sun et al. 2016). Sedanolid is a flavor-forming compound derived from celery seed oil. This compound functions as a suppressor of tumor cell viability and invite autophagy (Hsieh et al. 2015). Other compounds that have activity as antitumor Ar (+) Tumeron are included in the sesquiterpene group. Tumeron can inhibit the proliferation of cancer cells and trigger cell apoptosis (Nair et al. 2019). Some compounds which make dichloromethane extract of red edible canna have cytotoxic activity against colon cancer cells WiDr.

In conclusion, this study concludes that there are variations in the spectral pattern between the red and green edible canna cultivars in dichloromethane solvents. The differences that arise are due to differences in chemical bonds of compounds contained therein. The apparent variation is due to the difference between red and green cultivars in cytotoxic activity against WiDr colon cancer cells. Red edible canna rhizome dichloromethane extract is the most toxic of all the extracts tested. The compounds contained in the red edible canna rhizome extract can inhibit cancer cell proliferation and trigger cell death.

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