Cytotoxic activity of combined ethanolic extract of Cinnamomum Burmannii and Ocimum Tenuiflorum Linn against T47D cancer cells

Actividad citotóxica de una combinación del extracto etanólico de Cinnamomum Burmannii con Ocimum tenuiflorum Linn. contra las células cancerosas T47D

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Resumen

Cancer is one of the main causes of death in the world, including in Indonesia. Phytochemicals found in cinnamon bark and basil leaves can be used as alternative anti-cancer agents. This study was conducted to determine the cytotoxic activity of an ethanolic extract combination of *Cinnamomum burmannii* with *Ocimum tenuiflorum* Linn. against T47D cancer cells. Extraction of cinnamon bark and basil leaves was performed using ethanol 96%. Cinnamon bark was extracted by sonication, while basil leaves were extracted by maceration. The qualitative test of cinnamon bark was done with test tubes, while that of basil leaves used thin layer chromatography with silica gel GF_{254} for the stationary phase and *n*-hexane: ethyl acetate (7.5: 2.5) for the mobile phase. An MTT assay was used for the cytotoxicity test. Results from the qualitative methods showed that cinnamon bark contains phenols, quinones, and saponins, while basil leaves contain alkaloids, flavonoids, phenols, and terpenoids. The IC_{50} value of *Cinnamomum burmannii* was 465.21 µg/mL, while that of *Ocimum tenuiflorum* Linn. was 267.88 µg/mL. In the combinational cytotoxic test, effects were interpreted by the combination index (CI) value. The combinational cytotoxic test found that the Cinnamomum ethanolic extract (500 µg/mL) and that of Ocimum (500 and 50 µg/mL) together produced synergistic effects.

Keywords: *Cinnamomum burmannii*, *Ocimum tenuiflorum* Linn., MTT assay, combination.

Abstract

El cáncer es una de las principales causas de muerte en el mundo, incluso en Indonesia. Los fitoquímicos que se encuentran en la corteza de la canela y las hojas de albahaca se pueden utilizar como agentes anticancerígenos alternativos. Este estudio se realizó para determinar la actividad citotóxica de una combinación del extracto etanólico de Cinnamomum burmannii con Ocimum tenuiflorum Linn. contra las células cancerosas T47D. La extracción de corteza de canela y las hojas de albahaca se realizó con etanol al 96%. La corteza de canela se extrajo mediante sonicación, mientras que las hojas de albahaca se extrajeron mediante maceración. El ensayo cualitativo de la corteza de canela se realizó con probetas, mientras que el de hojas de albahaca utilizó cromatografía en capa fina con gel de sílice GF254 para la fase estacionaria y n-hexano: acetato de etilo (7,5: 2,5) para la fase móvil. Se utilizó un ensayo MTT para la prueba de citotoxicidad. Los resultados de los métodos cualitativos mostraron que la corteza de canela contiene fenoles, quinonas y saponinas, mientras que las hojas de albahaca contienen alcaloides, flavonoides, fenoles y terpenoides. El valor de CI50 de Cinnamomum burmannii fue de 465,21 µg/ mL, mientras que el de Ocimum tenuiflorum Linn. fue de 267,88 µg/mL. En la prueba citotóxica combinacional, los efectos se interpretaron mediante el valor del índice de combinación (IC). La prueba citotóxica combinada encontró que el extracto etanólico de Cinnamomum (500 µg/mL) y el de Ocimum (500 y 50 µg/mL) juntos produjeron efectos sinérgicos.

Palabras clave: Cinnamomum burmannii, Ocimum tenuiflorum Linn., Ensayo MTT, combinación.

Introduction

Cancer is a disease that is characterized by uncontrolled cell growth, invasion of local tissue, and the potential to metastasize[1]. Cancer is also one of the leading causes of death in the world. Based on data from the Ministry of Health of the Republic of Indonesia, the prevalence of cancer in the population of Indonesia in 2013 was 1.4%, or around 347,792 people. Breast cancer has the second-highest prevalence among women in Indonesia at about 0.5%[2].

Therapy for breast cancer can be administered through surgery (mastectomy), systemic therapy (chemotherapy), hormonal therapy, target therapy, or radiotherapy. Chemotherapy drugs are known to have side effects of hair loss, memory disorders, fatigue, and headaches[3]. The combination of chemotherapy agents is one strategy to make cancer therapy more effective and safe and to suppress the side effects of chemotherapy. Meanwhile, phytochemicals have the potential to be developed as anti-cancer agents[4], including basil (*Ocimum tenuiflorum*) and cinnamon (*Cinnamomum burmannii*).

Cinnamon displays anti-cancer activity with various mechanisms such as anti-proliferation, induction of cell death, anti-angiogenesis, anti-metastasis, suppression of tumor-triggered inflammation, immunomodulation, and modulation of redox homeostasis[5]. The methanol extract of cinnamon also exhibits cytotoxic activity against C666-1 nasopharyngeal cancer cells with an IC_{50} value of 30.67 ± 1.12 µg/mL[6]. In addition, cinnamon essential oil shows a synergistic effect with doxorubicin. In *in vitro* testing, it was found that the IC_{50} of cinnamon essential oil versus T47D breast cancer cells was 75 µg/mL [7,8].

Ethanol extract of basil leaves inflicts cytotoxic activity on MCF-7 breast cancer cells with an IC_{50} of 6.95 µg/mL[9]. The main chemical compounds found in basil leaves are flavonoids, oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and beta-caryophyllene[9]. The cytotoxic activity of basil leaves is caused by the content of apigenin, eugenol, luteolin, and ursolic acid[10]. This research aims to determine the cytotoxic activity of the combined ethanol extracts of cinnamon (*Cinnamomum burmannii*) and basil leaves (*Ocimum tenuiflorum* Linn.) on T47D breast cancer cells.

Methods

Materials

This study utilized a maceration vessel, a Heidolph rotary evaporator, a Memmert water bath, a Branson sonicator, a funnel, an Iwaki 96-well plate, a centrifuge, an Ohaus analytical balance, a Socorex micropipette, an Olympus microscope, a Neubauer hemocytometer, a Maxi Mix II vortex mixer, a Pasteur pipette, a Binder CO_2 incubator, an Esco laminar flow cabinet, a BioTek ELISA reader, cinnamon (*Cinnamomum burmannii*), basil leaves (*Ocimum tenuiflorum* Linn.), T47D cells, dimethyl sulfoxide (DMSO), RPMI culture media, fetal bovine serum (FBS) 10% v/v, penicillin-streptomycin 2%, doxorubicin, ethanol 96%, MTT solution (20%) in phosphate-buffered saline (PBS), 0.025% trypsin, 10% sodium dodecyl sulfate (SDS) in 0.01N HCl, distilled water, a conical tube, a yellow tip, a blue tip, a white tip, and filter paper.

Extraction

The cinnamon was extraction with ultrasonic waves by inserting 2.5 grams of mashed cinnamon and 25 mL of ethanol into an Erlenmeyer flask, then dipping it into an ultrasonic tank containing water and detergent. The extraction process was carried out at a fixed intensity (60%) for 66 minutes. Next, the extract was filtered with paper before it was separated by solvent with the rotary evaporator and water bath until thick extracts were obtained[11].

The ethanol extract of basil leaves was prepared by maceration. One hundred grams of basil leaves were weighed, then soaked in 300 mL ethanol for one day before the crude extract was filtered[12]. After twice repeating the immersion and filtration, the remaining ethanol in the filtrate was dispersed with the rotary evaporator and placed in the water bath until a thick extract was obtained.

Qualitative Test of Cinnamon Ethanol Extract Phytochemicals by Tube

The phytochemical qualitative test of the cinnamon ethanol extract was adapted from that of Pratibha et al.[13], in which preliminary experiments were aimed at identifying the content of alkaloids, flavonoids, phenol, and quinones. Ten milligrams of the cinnamon ethanol extract were dissolved in 10 mL of distilled water, then exposed to ultrasonic waves for five minutes.

Alkaloids

Approximately 0.5 mL of ammonia acid and 0.5 mL of chloroform were added to 2 mL of the cinnamon ethanol extract. The chloroform layer was retrieved and received 0.5 mL of 2N HCl, then was strongly shaken to form two layers. The upper layer (acid) was divided into three parts put into different tubes. Mayer's and Dragendorff's reagents, each as much as 0.5 mL, were added to the first and second tubes respectively. Any resulting turbidity or white sediment in the first tube indicates that the sample contains alkaloids, and so do turbidity or orange deposits within the second tube. The blank third tube was used as a comparison.

Flavonoids

One milliliter of lead acetate was dropped into 2 mL of cinnamon ethanol extract to identify the presence of flavonoids, which is marked by the solution's tint shifting from yellow to green.

Phenols

Five drops of dilute $FeCl_3$ were added to 0.5 mL of cinnamon ethanol extract. If a blackish-blue or blackish green precipitate appears, the sample is proven to contain phenols.

Quinones

A half milliliter of 2N NaOH was dropped into 0.5 mL of cinnamon ethanol extract. Quinones are present in the sample if their color turns reddish.

Saponins

Two milligrams of thick cinnamon ethanol extract were dis-

solved in 5 mL of distilled water in a closed container, which was then shaken vigorously. The occurrence of saponins in the sample is confirmed by the formation of foam with a thickness of at least 1 cm.

Qualitative Test of Basil Leaf Ethanol Extract with Thin Layer Chromatography (TLC)

A 1% stock solution was made by dissolving 10 mg of each thick extract of cinnamon and basil leaves in 1 mL of ethanol 96%. The stock solution was bottled on a silica gel GF254 plate with an elution distance of 8 cm. The mobile phase for the ethanol extract of basil leaves consisted of n-hexane: ethyl acetate (7.5 : 2.5) in 10 mL. After the elution was finished, the plate was dried at room temperature then seen under visible light, UV254 and UV366. To see whether there was a qualitative phenolic, terpenoid, saponin, and flavonoid content in the extract, the plate was subsequently sprayed with $FeCl_3$, Dragendorff's, citroborat, and Anisaldehyde-H_2SO_4 reagents. After being sprayed with the latter two reagents, the plate was dried in an oven under a temperature of 100˚C for five minutes.

Making Test Solutions

The stock solution was prepared by dissolving 10 mg of cinnamon ethanol extract in 100 μL DMSO with the addition of an RPMI medium of up to 1000 μL. The stock solution was placed into a sterile Eppendorf tube and stored in the refrigerator. In a single cytotoxic test, solutions were made in a series of dilutions (1000, 500, 250, and 100 μg/mL) for the ethanol extract of cinnamon, while for the ethanol extract of basil leaves the levels were 500, 250, 100 and 50 μg/mL. The concentrations of cinnamon ethanol extract used in the combination cytotoxic test were 500, 125, 62.5, and 31.25 μg/mL, and those of the ethanol extract of 500 basil leaves were 250, 100, and 50 μg/mL. All solutions were treated aseptically in a cytotoxic safety cabinet.

Cytotoxicity Tests

Cytotoxicity tests were carried out after 80% of the cells were confluent. The cell media were discarded, then a series of concentrations that had been made were inserted into the wells. For the control of T47D breast cancer cells and RPMI media, each well was filled with 100 μL DMSO for solvent control and the same amount of doxorubicin for positive control before being kept for 24 hours in the CO_2 incubator. The MTT reagent was prepared for treatment at 0.4 mg/mL by combining 1 mL of MTT stock solution with 5 mg/mL PBS and diluting them with 10 mL of RPMI media. The cell media were removed, 100 μL of MTT reagent was added in each well, and the cells were re-incubated. After 2-4 hours, the cells were observed by microscope. If formazan crystals had been formed, 100 μL SDS was added into all wells. The 96-well plate was then wrapped in paper and incubated for 24 hours in dark conditions at room temperature. Subsequently, cell absorbance at the 96 wells was examined using the ELISA reader with wavelength (λ) set at 550 nm. Following the absorbance reading, the proportion of live cells was calculated and the IC_{50} values were analyzed on Microsoft Excel.

Data Analysis

In this study, the absorbance of the solvent control was lower than that of the cell control, and so the percentage of viable cells was determined through the formula:

$$\text{Percentage of viable cells} = \frac{(\text{Absorbance of treatment} - \text{Absorbance of media control})}{(\text{Absorbance of solvent control} - \text{Absorbance of media control})} \text{x} 100$$

(1)

Afterward, a scatter plot of log concentration vs. percentage of viable cells was created along with a subtype comparing pairs of values, and the regression line was drawn to establish the linear regression equation and define the parameter r therein. It transpired that the resulting r was greater than the r_{table} which signifies that the equation meets the standard for finding the IC_{50} value. In order to gain IC_{50}, the value in the linear regression equation was set at 50% and its corresponding x value was then identified to determine the anti-log of the concentration[14].

Analysis of the combination of the cinnamon and basil ethanol extracts used a Combination Index (CI) value, which serves to evaluate combinational treatment via the following formula:

CI = D1/Dx1 + D2/Dx2 (2)

D1 and D2 represent the extract concentrations used in the combinational intervention, whereas Dx1 and Dx2 are single treatment concentrations that give the same responses as D1 and D2. The CI value obtained was applied to measure the cytotoxic potential of the ethanol extract combination of cinnamon and basil leaves on T47D cells[15].

Results and discussion

extraction

The cinnamon (*Cinnamomum burmannii*) was procured from Tawangmangu, Karanganyar, Indonesia. The stem was first finely powdered, passing a 100 mesh screen and yielding 13.76% extract with reddish-brown color (Table 1). The basil leaves (*Ocimum tenuiflorum* Linn.) also came from the same location. The leaves were separated from the stem and flowers, then dried at 40˚C, powdered with a size of 40 mesh, and twice macerated using ethanol 96% solvent for 24 hours each. The outcome was 6.17% concentrated green extract (Table 1).

Table 1. The yields of cinnamon and basil leaf extracts

Plant	Crude sample	Thick extract	Yield
Cinnamon	25.01 g	3.44 g	13.76 %
Basil leaves	79.25 g	4.89 g	6.17 %

Analysis of Compound Content in Samples

Qualitative Test of Cinnamon Ethanol Extract Phytochemicals by Tube

The extract of cinnamon was tested for its compound content through phytochemical screening by tube because thin layer chromatography (TLC) did not provide the optimal mo-

bile phase. Pratibha et al.[13] stated that the ethanol extract of cinnamon (*Cinnamomum burmannii*) contains alkaloids, flavonoids, phenols, quinones, and saponins, whereas the qualitative phytochemical test of cinnamon ethanol extract in this research showed that it contained phenols, quinones, and saponins (Table 2).

Table 2. The phytochemical screening of cinnamon ethanolic extract (Cinnamomum burmannii)

Compound	Reagent	Result	Conclusion
Alkaloid	Mayer's	There was no white sediment	-
	Dragendorff's	There was no reddish-pink sediment	-
Flavonoid	Pb acetate	The solution did not become greenish-yellow	-
Fenol	$FeCl_3$	The solution became black	+
Quinon	NaOH 2N	The solution became reddish	+
Saponin		Foam was formed	+

Qualitative Test of Basil Leaf Ethanol Extract with Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was conduct to test the compound content of the basil leaf ethanol extract since this method is applicable for qualitative analysis of organic compounds, isolation of pure compounds from mixed compounds, and isolation of preparative compounds[16]. The stationary phase used was silica gel GF254 which has polar properties, while optimization of the mobile phase utilized 10 mL of n-hexane and ethyl acetate with a ratio of 7.5: 2.5.

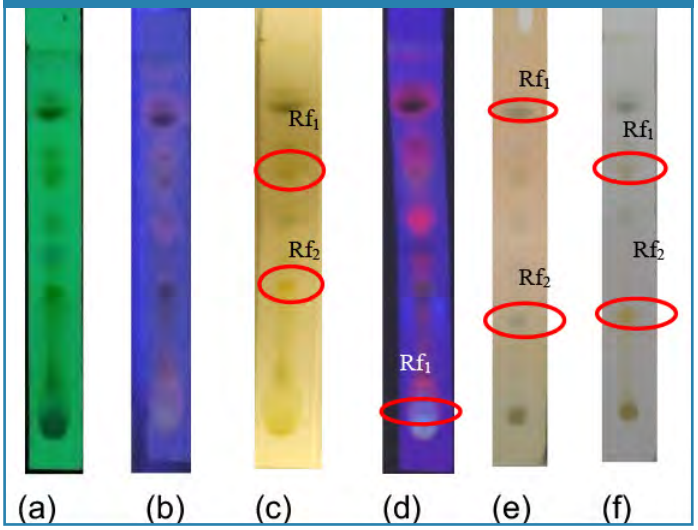
Visualization of the results of the separation of compounds by thin-layer chromatography can be clarified with spray reagents such as Dragendorff's, Anisaldehyde-H_2SO_4, $FeCl_3$, and citroborat. When sprayed with Dragendorff's reagent, positive spots turned brownish yellow, proving that the compounds included alkaloids that will generate a brown or brownish-yellow color shortly after being sprayed[17]. Meanwhile, spraying the citroborat reagent made the spots show blue fluorescence at UV366. A mixture of boric and citric acids, cytroboric acid is presumed to bind with flavonoid compounds when heated, producing fluorescent blue-green at UV366[6].

Compounds bearing polyphenols will display blackish gray spots after being sprayed with $FeCl_3$[17]. The positive ethanol extract of basil leaves incorporated polyphenol compounds, evidenced by black spots caused by phenolic group bonds that form a complex with $FeCl_3$ (Figure 1). In addition, compounds involving terpenoids will form yellow-brown, purple, and black colors in visible light if sprayed with Anisaldehyde-H_2SO_4[18]. In this study, positive results were confirmed by the emergence of yellow spots (Figure 1). Based on these outcomes, it can be summarized that the ethanol extract of basil leaves contained alkaloids, flavonoids, polyphenols, and terpenoids.

Table 3. Results of chemical compound detection in basil leaves

Reagent of identification	Positive result	Rf	Color	Compound
Dragendorff's	1 (Figure 2c)	Rf_1=0.66	Yellow	Alkaloid[17]
	2 (Figure 2c)	Rf_2=0.35	Brownish-yellow	Alkaloid[17]
Citroborat	1 (Figure 2d)	Rf_1=0.1	Blue	Flavonoid [17]
$FeCl_3$	1 (Figure 2e)	Rf_1=0.83	Black	Polyphenol[17]
	2 (Figure 2e)	Rf_2=0.29	Black	Polyphenol[17]
Anisaldehyde-H_2SO_4	1 (Figure 2f)	Rf_1=0.79	Yellow	Terpenoid[18]
	2 (Figure 2f)	Rf_2=0.63	Yellow	Terpenoid[18]

Figure 1. Results of TLC on basil leaf extract with n-hexane: ethyl acetate (7.2: 2.5) as mobile phase.



Detection of UV254 before spraying reagent (a), UV366 before spraying reagent (b), after spraying with Dragendorff's reagent in visible light (c), citroborat at UV366 (d), $FeCl_3$ in visible light (e), Anisaldehyde-H_2SO_4 in visible light (f).

Cytotoxic tests

Before cytotoxic testing, T47D cell cultures were first incubated for 24 hours to reach the log phase (characterized by 80% cofluent cells). Cytotoxicity tests were conducted using the MTT assay method. In viable cells, mitochondria propagate dehydrogenase which can break down the MTT tetrazolium ring and produce purplish-blue formazan crystals (Figure 3). If dehydrogenase is inactive due to cytotoxic effects, formazan crystals are not formed[9]. Formazan crystals are insoluble so it is necessary to add SDS 10% in 0.01N HCl, which dissolves salts, while the SDS 10% stops enzymatic reactions[13].

Figure 2. Formazan crystal formation mechanism[19]

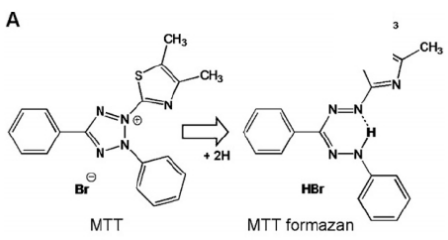


Figure 3. T47D cell morphology in cell control (a), cells treated with doxorubicin (b), cells with 500 μg/mL of basil leaf ethanol extract (c), cells treated with 500 μg/mL of cinnamon ethanol extract (d), cells with formazan crystals formed by MTT treatment (e). The arrows point to living cells (a), dead cells (b, c, d), and formazan crystals (e).

T47D cells, which express mutated p53 proteins, are commonly used in in vitro cytotoxic tests. Residue 194 undergoes missense mutation in the zinc-binding domain, L2, making p53 unable to bind to the response of the DNA element. This results in reduced or loss of p53's ability to regulate its cell cycle. T47D breast cancer cells are sensitive to the administration of doxorubicin[3-7]. Viable and dead cells have several morphological differences. In the control of viable T47D cells (Figure 4a), oval and transparent core cells were seen.

The positive control in this study utilized doxorubicin. The action mechanism of doxorubicin in T47D cancer cells is intercalating into DNA, thereby interfering with DNA formation mediated by topoisomerase II enzymes[6,7]. In the positive control, each dead cell is marked by a round shape and a black core (Figure 4b). Cell viability is presented in Table 4.

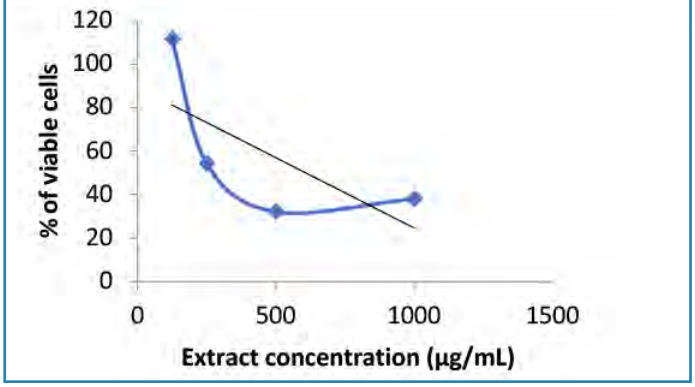
Table 4. Cell viability after treatment with doxorubicin (positive control)

Concentration	% of viable cells			
	I	II	III	Average
50 nM (0,272 μg/mL)	33.55	42.45	42.98	39.66
100 nM (0,543 μg/mL)	42.98	34.88	35.42	37.76

Table 5. Data after treatment with ethanolic extract of cinnamon on T47D cells

Concentration (μg/mL)	Log concentration	% of viable cells				SD
		I	II	III	Average	
125	2.09691	106.90	120.03	107.69	111.54	7.36
250	2.39794	52.77	54.76	55.43	54.32	1.8
500	2.69897	30.75	32.21	33.27	32.08	1.27
1000	3	38.18	37.78	38.05	38.01	0.20

Figure 4. Effect of cinnamon extract treatment on T47D cells.

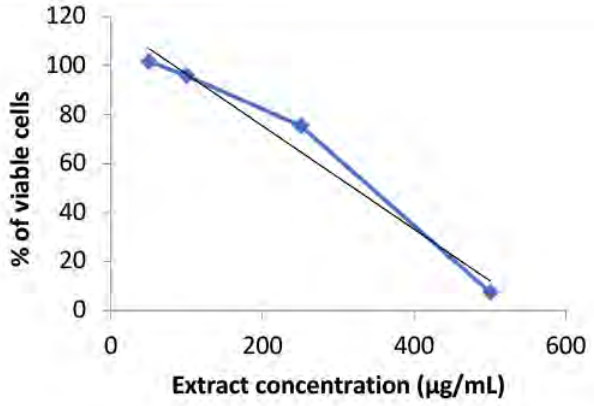


In Figure 5 it can be seen that viable cells had falling rates at concentrations between 125 and 500 μg/mL, but at 1,000 μg/mL the proportion slightly went back up. This demonstrates that the higher the concentration of the extract, the lower the percentage of viable cells, except at 1,000 μg/mL. In this study nonetheless, the increase in the concentration of cinnamon ethanol extract did not cause any decrease in the percentage of viable cells.

Table 6. Results of basil leaf ethanol extract treatment on T47D

Concentration (μg/mL)	Log concentration	% of viable cells				SD
		I	II	III	Average	
50	1.69897	102.92	97.35	104.91	101.72	3.92
100	2	94.03	94.16	99.07	95.76	2.87
250	2.9794	77.71	71.88	76.79	75.46	3.14
500	2.69897	7.54	5.81	9.00	7.45	1.59

Figure 5. Effect of basil leaf ethanol extract on T47D cells.



The effects of treatment with the ethanol extract of basil leaves can be viewed in Figure 6, which reveals that the extract concentration is inversely proportional to the percentage of living cells. This can be observed in the treatment with the lowest concentration (50 μg/mL) where the proportion of viable cells was 101.72%, while at the highest concentration (500 μg/mL) the rate was 7.24%, thus signifying that the cytotoxic activity of this basil leaf ethanol extract is a dose-dependent response.

Outcomes of this research disclosed that doxorubicin as a positive control could induce cell death, as indicated by less than 50% of viable cells in concentrations of 50 nM and 100 nM (Table 4). This implies that in order to reduce viable cells to below 50%, the minimum concentration of cinnamon ethanol extract was not needed while that of the ethanol extract of basil leaves was 500 μg/mL. Viable cell percentages of less than 50% after treatment with various concentrations of both extracts enabled the estimation of IC_{50} values with results given in Table 7.

Table 7. Cytotoxic test of cinnamon and basil leaf ethanol extracts

Treatment	IC_{50}
Ethanol extract of cinnamon	456.01 μg/mL
Ethanol extract of basil leaves	266.43 μg/mL

A chemical compound is a potent anti-cancer if its IC_{50} value does not reach 100 μg/mL, has moderate cytotoxic activity if the IC_{50} stands between 100 and 1,000 μg/mL, and has no

cytotoxic activity if the IC_{50} exceeds 1000 μg/mL[16]. Based on this categorization, it can be stated that the ethanol extracts of cinnamon and basil leaves have moderate cytotoxic activity.

Ethanol extract of cinnamon has moderate cytotoxic activity against T47D breast cancer cells with an IC_{50} of 456.01 μg/mL, compared to that of cinnamon essential oil at 75 μg/mL in a previous study by Anjarsari et al[7]. In addition, other studies have found that cinnamon essential oil can prompt apoptosis in T47D breast cancer cells[5]. Dissimilarities in these results may be caused by differences in the source of the sample, in that cinnamon essential oil contains more trans-cinnamaldehyde, the main component in cinnamon, than the extract. This suggests that the compound plays a central role in their cytotoxic activity[14-17].

Cinnamon ethanol extract includes polyphenols, quinones, and saponins as detected through qualitative phytochemical tests using the tube method (Table 2). Possible compounds that influence cytotoxic activity in cinnamon are trans-cinnamaldehyde and other compounds, such as polyphenols in the extract which exhibit a synergistic effect in cytotoxicity tests[7]. Trans-cinnamaldehyde is a derivative of cinnamic acid, which belongs to the phenolic group. The mechanism of trans-cinnamaldehyde in cytotoxic activity augments the production of reactive oxygen species (ROS) in cancer cells, inhibits NF-kB transcription activity, and depletes the transmembrane potential of mitochondria[5].

Ethanol extract of basil leaves has a moderate cytotoxic effect on T47D with an IC_{50} of 266.43 μg/mL, whereas such extract in prior research produced IC_{50} values of 6.95 μg/mL and 176.37 μg/mL on MCF-7 cells[8,9]. When tested on T47D cells, the ethanol extract of basil leaves at a concentration of 500 ppm gave the smallest percentage of living cells at 58.582%, indicating that the extract was more potent against T47D than others in the past studies. This may be attributed to disparities in variability (place of sampling) that might have affected the characteristics of the sample.

The synergy of the compounds in the components of basil leaves can provide cytotoxic activity. Basil leaves contain ursolic acid and eugenol[10] which are terpenoid compounds[18]. Meanwhile, two types of polyphenols found in basil leaves are orientin and vicentin which are radioprotective flavonoids, in that they counteract hydroxyl radical compounds that increase free radicals in cells[12]. The alkaloids, flavonoids, polyphenols, and terpenoids in the basil leaf ethanol extract were identified in the qualitative test of compound content by TLC (Table 3, Figure 2).

The cytotoxic activity of combined cinnamon and basil leaf ethanol extracts was examined by testing the combination with varied extract concentrations (Table 8), which were brought together randomly to determine the optimal concentrations that produce synergistic cytotoxic activity.

Table 8. Concentrations of combined cinnamon and basil leaf ethanol extracts

Concentration 1		Concentration 3	
Basil leaves (µg/mL)	**Cinnamon (µg/mL)**	**Basil leaves (µg/mL)**	**Cinnamon (µg/mL)**
50	31.25	50	125
100		100	
250		250	
500		500	

Concentration 2		Concentration 4	
Basil leaves (µg/mL)	**Cinnamon (µg/mL)**	**Basil leaves (µg/mL)**	**Cinnamon (µg/mL)**
50	62.5	50	500
100		100	
250		250	
500		500	

The viability of T47D cells in the combination treatment was estimated based on absorbance data. Lower viability indicates greater potency of the combination of extracts in inducing cell death[15]. Table 9 describes the results of cell viability calculations.

Table 9. Cell viability of T47D in combination test of cinnamon and basil leaves

		Basil leaves				Average of viable cells
	Concentration (µg/mL)	**500**	**250**	**100**	**50**	
Cinnamon	**500**	**66.17**	94.30	102.52	96.20	89.80
	125	99.43	99.43	107.92	108.67	103.86
	62.5	77.58	106.59	123.79	131.48	109.86
	31.25	88.64	109.77	130.16	134.62	115.80
Average		70.84	82.95	102.52	116.10	117.74

Outcomes of analysis on the combination of cinnamon ethanol extract with that of basil are presented in combination index (CI) values as provided in Table 10. These results were then interpreted with synergistic values in Table 11.

Table 10. Combination index values

CI	Basil leaves				
	Concentration (µg/mL)	**500**	**250**	**100**	**50**
Cinnamon	500	**0.51***	3.65	1.12	**0.37***
	125	4.56	4.26	3.10	2.82
	62.5	1.08	4.65	7.36	6.59
	31.25	2.24	7.39	9.78	7.51

*has synergistic effect

Table 11. Combination index value interpretation[15]	
CI value	Interpretation
<0.1	Very strong synergistic effect
0.1-0.3	Strong synergistic effect
0.3-0.7	Synergistic effect
0.7-0.9	Light-medium synergistic effect
0.9-1.1	Approaching additive effect
1.1-1.45	Mild-moderate antagonist effect
1.45-3.3	Strong antagonistic effect
>3.3	Very strong antagonistic effect

According to the interpretation of the combination index, the ethanol extract of cinnamon with a concentration of 500 μg/mL is synergistic with that of basil leaves at 500 and 50 μg/mL. Cinnamon ethanol extract gave an additive effect at 62.5 μg/mL when combined with 500 μg/mL of basil leaf ethanol extract, while at other concentrations the combination had mild to strong antagonistic effects.

Synergism is an interaction that produces a greater effect than a combination of two different compounds rather than a single compound[2,3]. In this study, results show that when combined, not all concentrations of cinnamon ethanol extract have synergistic cytotoxic activity with the ethanol extract of basil leaves. This may due to inadequate combination concentrations to kill T47D breast cancer cells, thus giving an antagonistic effect between both extracts. Nevertheless, the combination of cinnamon and basil leaf ethanol extracts did synergize, leading to the conclusion that the combination potentially improves the cytotoxic activity in T47D cells. In the future, it is probably necessary to try other cinnamon extraction solvents or replace the samples with essential oil to obtain better cytotoxic activity, and to test the combination of the two extracts on other cells which are more potential for cytotoxicity tests such as MCF-7 cells.

Conclusion

Based on the results of this research, it can be concluded that the combined ethanol extracts of cinnamon (*Cinnamomum burmannii*) and basil leaves (*Ocimum tenuiflorum* Linn.) demonstrate cytotoxic activity against T47D cells with IC_{50} values at 456.02 and 266.43 μg/mL, respectively. The chemical compounds uncovered by the qualitative testing of cinnamon were phenols and quinones, whereas those in basil leaves were alkaloids, flavonoids, phenolics, and terpenoids. The combination of the two extracts produced a synergistic effect at a concentration of 500 μg/mL of cinnamon ethanol extract and 500 and 50 μg/mL of basil leaf ethanol extract.

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