Science and Technology Indonesia
e-ISSN:2580-4391 p-ISSN:2580-4405
Vol. 11, No. 2, April 2026

Research Paper

Anticancer Efficacy of a Combination of *Curcuma longa* L. Rhizome and *Annona muricata* L. Leaf Extracts Against T47D Cells

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Abstract

Breast cancer remains one of the most prevalent malignancies worldwide. The use of combinations of natural products is increasingly recognized as a promising strategy for cancer treatment. Turmeric rhizome (*Curcuma longa* L.) and soursop leaves (*Annona muricata* L.) are two natural materials known for their anticancer potential. This study aimed to identify the phytochemical constituents of turmeric rhizome and soursop leaf extracts and to evaluate the anticancer activity of their combination against T47D breast cancer cells. Turmeric and soursop leaves were extracted with 96% ethanol using the maceration method. Raw material standardization was performed by measuring water content, ethanol-soluble content, and water-soluble content. The extract was standardized by thin-layer chromatography. T47D and Vero cell lines were used in this study. Compound identification was performed using Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (LC/Q-TOF-MS). The candidate combination ratio was determined using the simplex lattice design approach in Design Expert 13 software. Cytotoxicity and antiproliferative effects were assessed using the MTT assay, while antimetastatic potential was evaluated through the scratch assay. Apoptosis and cell cycle arrest were analyzed by flow cytometry. IC_{50} data were analyzed using one-way ANOVA, and post hoc testing was performed using Tukey's multiple-comparison test. Antiproliferation and scratch assay data were analyzed using two-way ANOVA followed by the Bonferroni test. Apoptosis and cell cycle assay data were analyzed using one-way ANOVA followed by the Tukey post hoc test. Phytochemical profiling indicated the existence of fifteen chemicals in both turmeric rhizome and soursop leaf extracts. The candidate combination ratio of turmeric rhizome to soursop leaf extracts was 1:21, exhibiting cytotoxic activity with an $IC50$ value of 32.2 ± 2.5 μg/mL. The combined extract was associated with antiproliferative, anti-migratory, and pro-apoptotic responses and induced G2/M phase cell cycle arrest in T47D cells.

Keywords

Anticancer, Combination, Phytochemical Compounds, Soursop Leaf Extract, Turmeric Rhizome Extract

Received: 20 October 2025, Accepted: 15 January 2026
https://doi.org/10.26554/sti.2026.11.2.405-419

1. INTRODUCTION

Cancer is a disease resulting from alterations in signaling and metabolic pathways that lead to uncontrolled cell proliferation and changes in cells (Kania et al., 2025; Rukmana et al., 2017). Among various types of cancer, breast cancer remains one of the most prevalent and life-threatening malignancies affecting women worldwide. Its global incidence continues to rise, with approximately 2.3 million new cases diagnosed annually (Dhefer, 2025). In 2020, more than 685,000 women died from breast cancer, accounting for roughly 16% (or one in every six) female cancer-related deaths (Arnold et al., 2022).

The increasing prevalence of breast cancer is associated with multiple risk factors, including (a) hormonal influences, (b) reproductive history, (c) genetic predisposition, (d) age, (e) lifestyle, (f) nutritional habits, and (g) exposure to ionizing radiation (Smolarz et al., 2022). Conventional treatment strategies, such as chemotherapy and radiotherapy, remain the mainstay for managing various stages of breast cancer. However, repeated exposure to these therapies often leads to the development of drug resistance, rendering cancer cells less responsive to treatment (Febriani et al., 2024). Moreover, chemotherapy lacks selectivity, affecting not only cancerous but also healthy cells, which contributes to adverse effects such as nausea, vomiting, fatigue, appetite loss, taste alterations, alopecia, xerostomia, and constipation (Aseeri et al., 2013). In recent years, increasing attention has been focused on natural compounds with potential anticancer activity (Prastiyanto et al., 2020; Rukmana et al., 2016). Two promising natural sources in this regard are turmeric rhizomes (*Curcuma longa* L.) and soursop leaves (*Annona muricata* L.). Previous studies have demonstrated that curcumin, the principal bioactive compound in turmeric, can inhibit cellular proliferation, decrease cell viability, and induce apoptosis in both human and animal breast cancer models (Barcelos et al., 2022; Sager et al., 2024). Similarly, soursop leaf extract has been reported to reduce tumor size and weight, exhibit antimetastatic activity, and induce apoptosis in breast cancer cells. Despite numerous studies on each plant separately, evidence on the anticancer efficacy of combining the two plants remains limited (Syed Najmuddin et al., 2016).

Mechanistically, curcumin suppresses the STAT3 and NF-κB signaling pathways, both of which play pivotal roles in cancer progression. Inhibition of these pathways suppresses of breast cancer cell proliferation, tumorigenesis, and metastasis (Farghadani and Naidu, 2021). Curcumin exhibits significant cytotoxicity across various breast cancer cell lines, including T47D (IC_{50} = 2.07 μM), MCF-7 (IC_{50} = 1.32 μM), MDA-MB-231 (IC_{50} = 11.32 μM), and MDA-MB-468 (IC_{50} = 18.61 μM) (Hu et al., 2018). Similarly, ethanolic extracts of soursop leaves inhibit the proliferation of T47D cells in vitro and show high cytotoxicity toward MCF-7 cells, accompanied by alterations in cell morphology and mRNA expression profiles (Fertilita et al., 2020; Hadisaputri et al., 2021). Combination therapy is extensively employed in breast cancer treatment to mitigate route redundancy and combat treatment resistance; likewise, the amalgamation of bioactive natural ingredients may provide multi-target benefits while potentially decreasing the necessary dosages (Hermansyah et al., 2023; Silfarohana et al., 2025).

Soursop leaf extract can inhibit the activity of the COX-2 (cyclooxygenase-2) enzyme, thereby diminishing EGFR signaling and reducing the proliferation and metastasis of cancer cells (Ilango et al., 2022). In parallel, turmeric extract has been shown to suppress STAT3 and NF-κB signaling pathways, both of which promote tumorigenesis and inhibit apoptosis (Thongrakard et al., 2014). Due to the functional interconnection between inflammatory transcription factors (STAT3/NF-κB) and growth factor signaling (COX-2/EGFR axis) in promoting proliferation and survival, concurrent inhibition of these pathways offers a credible molecular foundation for additive or synergistic cytotoxicity. These two characteristics may complement each other and provide a foundation for exploring combination medicines that enhance anticancer efficacy. Nonetheless, the combined effect of the two extracts may also yield an antagonistic interaction. Consequently, the impact of combination therapies must be quantitatively assessed (Donkor et al., 2023). This study assessed the combined effect of turmeric and soursop leaf extracts on the improvement of anticancer activity, specifically evaluating cytotoxicity in T47D cells using IC50 values. To facilitate interpretable interaction analysis, a 1:21 (turmeric:soursop) ratio was chosen based on preliminary IC_{50} screening in T47D cells, where this ratio produced the lowest observed IC_{50} value. Accordingly, this study aimed to evaluate the anticancer activity of a candidate combination of turmeric rhizome and soursop leaf extracts against T47D breast cancer cells and to qualitatively identify its bioactive constituents using Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC/Q-TOF-MS).

2. EXPERIMENTAL SECTION

2.1 Material

The study was performed at the standardization laboratory for traditional medicine raw materials, Tawangmangu, under Indonesia's National Research and Innovation Agency (BRIN). Turmeric was sourced from farmers at the medicinal plant center in Karanganyar, Central Java, Indonesia, while soursop leaves were procured from medicinal plant farmers in Boyolali, Central Java, Indonesia. The Laboratory of Traditional Medicine Raw Materials, BRIN, Tawangmangu, supplied T47D ATCC HTB-133 and Vero ATCC CCL-81 cell lines. Fetal Bovine Serum (FBS), dimethyl sulfoxide, phosphate-buffered saline, DMEM powder, MTT reagent, Annexin V-FITC, and flow cytometry reagents were acquired from Sigma Aldrich, St. Louis, Missouri. Filter paper and ethanol were procured from Merck Life Science in Darmstadt.

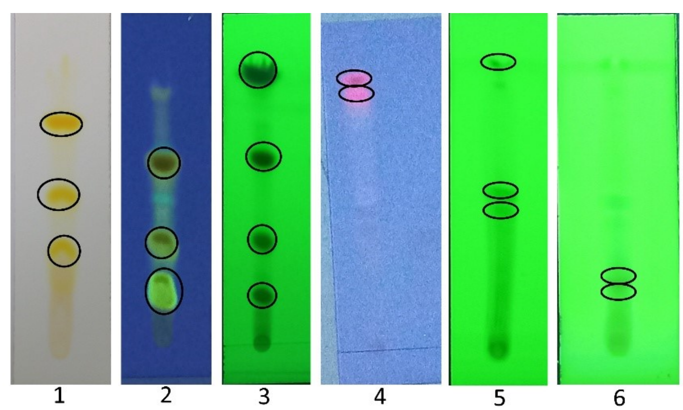

Figure 1. Thin-Layer Chromatography Profiles of Turmeric and Soursop Leaf Extracts: 1. Flavonoids in Turmeric Extract After Spraying with Sitroborate; 2. Phenolics in Turmeric Extract After Spraying with $FeCl_3$; 3. Alkaloids in Turmeric Extract After Spraying with Dragendorf; 4. Flavonoids in Soursop Leaf Extract After Spraying with Sitroborate; 5. Phenolics in Soursop Leaf Extract After Spraying with $FeCl_3$; 6. Alkaloids in Turmeric Extract After Spraying with Dragendorf

2.2 Sample Preparation

Turmeric and soursop were identified at the Traditional Medicine Raw Materials Standardization Laboratory of the National Research and Innovation Agency (BRIN) in the Tawangmangu Region. Turmeric rhizomes and soursop leaves were sorted, washed with running water, and chopped. Afterward, the turmeric rhizomes and soursop leaves were dried in an oven for 3 × 24 hours at 40 °C. Each sample was mechanically ground using a grinding machine to obtain a fine powder. Each simplicia was sieved using a 40-mesh sieve. The water content of each sample was calculated using a moisture analyzer. The ethanol-soluble and water-soluble extract content of each sample was measured (Dwi et al., 2024).

2.3 Turmeric Rhizome and Soursop Leaf Extraction

Each sample of turmeric rhizomes and soursop leaves was cleaned, dried, and ground to obtain fresh herbal extracts. Five hundred grams of each herbal extract were weighed and then extracted with 96% ethanol for 3 × 24 hours. The filtrate was then filtered through filter paper to obtain a macerate. The resulting macerate was dried and concentrated using a rotary evaporator at 45 °C (Maharani et al., 2024). The extraction yield was calculated, and the organoleptic assay was performed.

2.4 Identification of Compound Groups in Turmeric and Soursop Leaf Extracts

Identification of compound groups in the extracts was performed using thin-layer chromatography (TLC). The stationary phase used was a silica gel 60 F_{254} plate, while the mobile phase for turmeric extract was chloroform:methanol (95:5). The mobile phase for soursop leaf extract was ethyl acetate:methanol:ddH_2O (15:3:2). Compound identification was conducted using spray reagents, including sitroborate for flavonoid identification, $FeCl_3$ spray reagent for phenolic identification, and Dragendorff spray reagent for alkaloid identification (Rukmana et al., 2017).

2.5 Metabolite Profiling and Qualitative Compound Identification by Liquid Chromatography Quadrupole/Time-of-Flight Mass Spectrometry

Turmeric rhizome and soursop leaf extracts were screened for phytochemical qualitative compounds using Liquid Chromatography Quadrupole/Time-of-Flight Mass Spectrometry (LC/Q-TOF-MS). The instrument used was an Agilent Revident 1290 Infinity II with a ZORBAX Eclipse Plus C18RRHD column (2.1 × 50 mm × 1.8 μm). The mobile phase consisted of water (A) and acetonitrile (B), containing 0.1% formic acid with a flow rate of 0.300 mL/minute. Gradient elution was as follows: 0-0.5 min 98% A, 0.5-13 min 98% A to 5% A, 13-13.8 min 5% A, 13.8-14 min 5% A to 98% A, and 14-18 min 98% A. The required sample injection was 10 μL, and the column temperature was 30 °C. ESI was set to positive mode, and monitoring was performed over a mass range of m/z 50-1200 (Oh et al., 2024).

2.6 Formulation of the Combination of Turmeric Rhizome Extract and Soursop Leaf Extract

The combination of turmeric rhizome extract and soursop leaf extract was performed using the simplex lattice design method using Design-Expert® software version 13 (Stat-Ease Inc., USA). The simplex lattice design approach was used to optimize the combination ratio of turmeric extract and soursop leaf extract for cytotoxicity assay against T47D cells. The simplex lattice design generated six mixture formulations (Table 1). The total concentration of the mixture was sustained at 100 μg/mL. Conversely, only the proportions of each component were modified inside the mixture space (turmeric extract + soursop leaves extract = 100%). Each proposed mixture was prepared by combining the extracts according to the proportions generated by the program and subsequently evaluated in vitro using the MTT assay. The IC_{50} was analyzed using a linear mixture model in Design-Expert. In a two-component mixture, the model is represented as Y = b1A + b2B, where A and B are the proportions of turmeric and soursop extracts, respectively. Model adequacy was evaluated using ANOVA ($p < 0.05$), a lack-of-fit test, and goodness-of-fit metrics (R^2, adjusted R^2, and predicted R^2). The candidate combination was determined by numerical optimization to minimize IC_{50}, and the three formulations with the lowest anticipated IC_{50} values underwent confirmation using the MTT assay. The candidate combination formula was chosen due to its relatively lower IC_{50} value among the combinations assessed under in vitro experimental settings. (Berlin et al., 2023).

2.7 Utilizing the MTT Assay Method for Cytotoxic Activity

T47D and Vero cells were thawed and cultured in DMEM media and then incubated in a 5% CO_2 incubator at 37 °C. Cells that were 80% confluent were harvested, and the cells were ready for cytotoxic assay (Djamal et al., 2020; Seran et al., 2020). The cytotoxicity assay of the combination extract of turmeric rhizome and soursop leaves was performed using a 96-well plate. Each well was seeded with 1 × 104 cells in 100 μL of medium. The microplate was incubated at 37 °C, 5% CO_2, and 95% humidity for 24 hours (Rukmana et al., 2025). Subsequently, each well received 100 μL of the combination extract at concentrations of 250, 125, 62.5, 31.25, 15.625, 7.813, and 3.906 μg/mL, determined using simplex lattice design (Table 1). The positive control consisted of varying concentrations (100, 50, 25, 12.5, 6.25 μg/mL) of doxorubicin. Each treatment was performed in triplicate. The negative control wells contained 100 μL of culture medium, while the solvent control wells contained 100 μL of 0.5% DMSO. After incubation, MTT (100 μL at 0.5 mg/mL) was added to each well, followed by 100 μL of 10% SDS stopper reagent in 0.1 N HCl. The results were read using an ELISA reader at a wavelength of 595 nm (Ifandari et al., 2020). The IC_{50} value was obtained by first calculating the percentage of cell viability using Equation 1 (Widiyastuti et al., 2019).

$$\text{Cell viability (\%)} = \frac{(\text{sample absorbance} - \text{media control absorbance})}{(\text{cell control absorbance} - \text{media control absorbance})} \times 100\% \quad (1)$$

Table 1. Combination of Turmeric Rhizome Extract and Soursop Leaf Extract

Combination type	Combination ratio	
	Turmeric rhizome extract	Soursop leaf extract
1	1	0
2	1	1
3	0	1
4	1	0
5	1	3
6	3	1

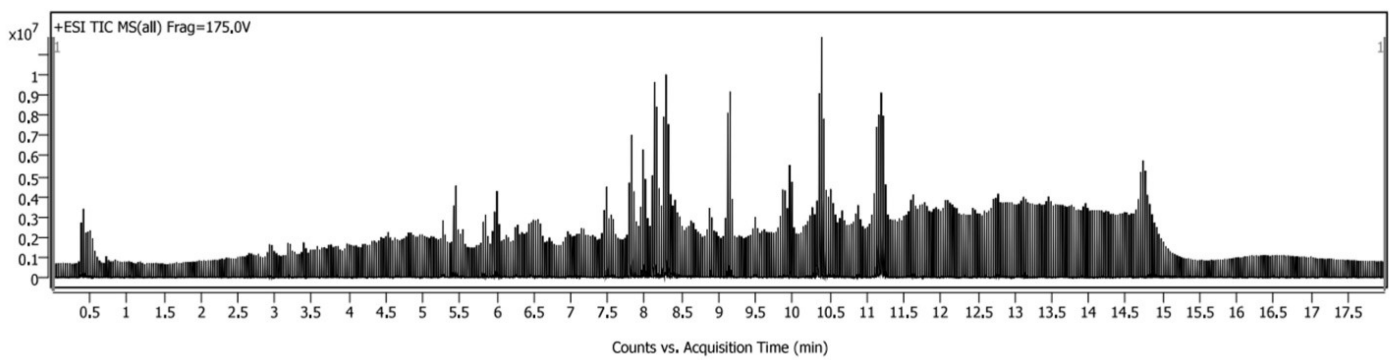

Figure 2. Total Ion Chromatogram of the Turmeric Rhizome Extract Identified by LC/Q-TOF-MS

2.8 Antiproliferation Assay of the Candidate Combination of Turmeric Rhizome and Soursop Leaf Extracts on T47D Cells

The combination of turmeric rhizome and soursop leaf extracts with the lowest IC_{50} value was selected as a candidate combination. The cytotoxicity test results for the candidate combination of turmeric rhizome and soursop leaf extracts on T47D cells were followed by an antiproliferation test. The antiproliferation test of the candidate combination of turmeric rhizome and soursop leaf extracts on T47D cells was conducted using the MTT assay with incubation times ranging from 0 to 24, 48, 72, and 96 hours. The candidate combination extract concentrations used as an antiproliferation assay were ¼, ½, 1, and $2 \times IC_{50}$ (Pratiwi et al., 2019).

2.9 Antimetastasis Test for the Candidate Combination Extract Against T47D Cells Using the Scratch Assay Method

The antimetastasis test of the candidate combination extract was performed using the scratch assay. assay method. T47D cells were cultured using 24-well plates (density of 125×103 cells/well), with a culture media volume of 500 μL/well. After the cell density reached approximately 90%, the cells were scratched and the complete culture media was replaced with new culture media (starvation media). Cell scratching was carried out using a yellow-tip and continued with washing the cells using 500 μL of PBS. The next stage was that the cells were treated with the best combination extract with a series of concentrations of ½, 1, and $2 \times IC_{50}$. In addition, control cell treatments and doxorubicin positive controls were also created. The treatment used starvation media with a total of 1000 μL/well. Next, the cells were incubated, observed, and documented at 0, 24, and 48 hours. Finally, data analysis was carried out using ImageJ to obtain the % cell coverage area value. The formula for the percentage of coverage area is shown in Equation 2 (Krisnayanti et al., 2024; To'bungan et al., 2022).

$$\text{Percent area of closure} = \frac{\%\text{ area of closure } t_n \text{ (incubation time to } n)}{\%\text{ area of closure } t_0 \text{ (incubation time to 0)}} \quad (2)$$

2.10 The Apoptosis Assay for the Candidate Combination Extract Against T47D Cells Used Flow Cytometry

Candidate combination extracts were created at concentrations of ½, 1, and 2× the IC_{50}. The T47D cell culture used was 2000 μL at a density of 5×10 cells/mL medium, distributed into 6-well plates and incubated for 24 hours in an incubator (370 °C, 5% CO_2, 95% humidity). After incubation, the cells were washed with 1000 μL of 1X PBS and treated with the candidate combination of extracts. Control cells were added with 2000 μL of culture medium, while positive controls were added with 2000 μL of doxorubicin at the IC_{50} concentration. Cells were incubated for 48 hours, harvested using 150 μL of 0.25% trypsin-EDTA and 1000 μL of media, then resuspended. The next step was to add 100 μL of binding buffer and 10 μL

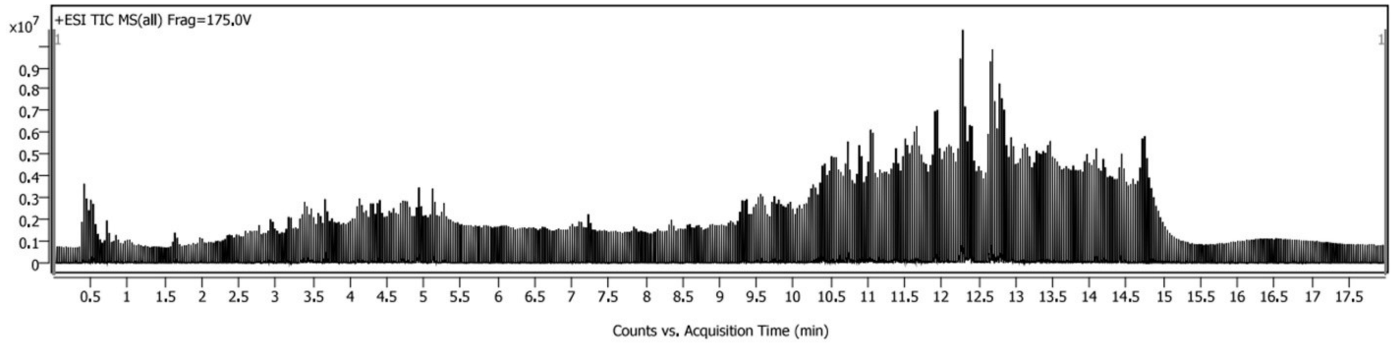

Figure 3. Total Ion Chromatogram of the Soursop Leaf Extract Identified by LC/Q-TOF-MS

of Annexin V-FITC to the cells. Afterwards, the cells were incubated in the dark for 15 minutes at room temperature, washed using binding buffer, and then 5 μL of propidium iodide solution was added before being directly analyzed using FACS Calibur flow cytometry (Gajate et al., 2021; Kouroshnia et al., 2022).

2.11 Cell Cycle Arrest Test of the Best Extract Combination on T47D Cells Using Flow Cytometry

2000 μL of T47D cells at a 5 × 105 density were distributed into six-well plates and incubated for 24 hours in an incubator (37 °C, 5% CO_2, 95% humidity). Next, the candidate combination of extracts was given with concentrations of ½, 1, and 2 × IC_{50}. While the control cells were added with 2000 μL of culture media, for the positive control, 2000 μL of doxorubicin with an IC_{50} concentration was added. After treatment, the cells were incubated again for 24 hours (Rukmana et al., 2017). Next, the cells were harvested by adding 150 μL of 0.25% trypsin-EDTA, and 1000 μL/well of culture media was added, resuspended, and transferred into a conical tube. Next, the cells were added with 500 μL of 70% cold ethanol to fix the cells. Next, the tube was stored at 37 °C for 30 minutes. The conical tube was centrifuged at 2000 rpm for 3 minutes, then the supernatant was discarded. The tube was wrapped with aluminum foil, and flow cytometry reagents were added (25 μL PI + 1 μL RNAase + 0.5 μL Triton-X + 500 μL PBS for 1 sample) and left for 30 minutes (Kouroshnia et al., 2022). The final stage was that the cell suspension was left again and transferred into a flow cytometry tube, then analyzed using FACS Calibur flow cytometry. The flow cytometer is used to see the percentage of the cell population in various cell cycle phases (Şimşek et al., 2020).

2.12 Analysis of Data

Cell viability calculations and IC_{50} values were performed using MS Office Excel 2021. The IC_{50} values were derived from dose–response data and analyzed across groups using one-way ANOVA, followed by Tukey's multiple comparisons test (α = 0.05). Antiproliferation data were analyzed using two-way ANOVA, with concentration, time, and their interaction as factors, followed by Bonferroni test at each designated time point. Scratch assay data were subjected to a log transformation [log10(x+1)] before analysis to satisfy the normality and homogeneity assumptions better. Scratch assay data were analyzed using a two-way ANOVA with concentration, time, and their interaction as factors. Subsequent evaluations were conducted using Bonferroni test at each time interval. Flow cytometry data from the Statistical analyses were conducted utilizing IBM SPSS Statistics 25, with α = 0.05 being statistically significant (Widiyastuti et al., 2018).

3. RESULT AND DISCUSSIONS

3.1 Sample Extraction Results

Turmeric and soursop leaves were standardized by plant identification, quality inspection of the medicinal plants, particle size (40-mesh sieve), calculation of water content, and determination of ethanol-soluble and water-soluble extract content (Table 2). The results of the compound identification of turmeric and soursop leaf extracts are shown in Figure 1. Compound identification was carried out for several groups of compounds, including phenolics, flavonoids, and alkaloids.

Extraction of turmeric rhizomes and soursop leaves was carried out using 96% ethanol as a solvent with a 1:10 ratio of the sample to the solvent. The resulting yields from the extraction process were 24.39% and 18.93%, respectively (Table 3). Based on organoleptic testing, the turmeric rhizome extract had a coagulated form and a deep yellow color. Meanwhile, organoleptic tests on soursop leaves showed the extract to be thick and dry, with a brownish-green color. Table 3 provides complete data on the extraction results.

3.2 Compound Identification Results in the Extract Using Liquid Chromatography Quadrupole/Time-of-Flight Mass Spectrometry (LC/Q-TOF-MS)

Metabolite profiling and qualitative compound identification of turmeric extract and soursop leaf extract were conducted using the Liquid Chromatography Quadrupole/Time-of-Flight

Table 2. Standardization of Quality Inspection of Turmeric and Soursop Leaf Medicinal Plants

Type of crude drug	Type of examination			
	40-mesh sieve	Moisture content	Ethanol-soluble extractive content	Water-soluble extractive content
Turmeric crude drug	✓	4.39 ± 0.59	21.10 ± 1.97	17.46 ± 3.59
Soursop leaf crude drug	✓	6.30 ± 0.54	17.17 ± 3.17	18.70 ± 1.69

Table description: ✓ = 40-mesh sieve

Table 3. Results of Turmeric Rhizome and Soursop Leaf Extract Yields

Sample	Comparison (Sample:Solvent)	Powder Weight (g)	Extract Weight (g)	Yield (%)
Turmeric rhizome	1:10	500	121.94	24.39
Soursop leaves	1:10	500	94.64	18.93

Mass Spectrometry (LC/Q-TOF-MS) technique. The LC-QTOF-MS analysis in this study used precise mass and retention time data. Validation of metabolite profiles and qualitative identification of compounds are conducted through accurate mass, database comparison, retention time analysis, formula verification, CAS (Chemical Abstracts Service) reference, matching score (Score/Score DB), and mass error assessment (Diff (DB, ppm)). The LC/Q-TOF-MS results of the turmeric rhizome extract showed that there were 15 identified compounds. The total ion chromatogram of the turmeric rhizome extract showed a relatively rich spectrum with various small peaks. This indicates that the sample is very complex (Figure 2). Table 4 shows the various compounds qualitatively identified in the turmeric rhizome extract. LC/Q-TOF-MS analysis of turmeric extracts revealed that most candidate compounds demonstrated elevated database match scores and score (DB) exceeding 95, with discrepancies in measured mass relative to database mass ranging from -0.06 to 0.25, indicating precise mass alignment under the specified analytical conditions.

Table 4 shows that LC/Q-TOF-MS identified 15 candidate compounds. Of these 15 candidate compounds, curcumin was found to be a member of the curcuminoid group. LC/Q-TOF-MS results do not indicate the abundance of phytochemical compounds, but only show the results of compound identification along with the characteristics of the identified compounds. Curcumin compounds can inhibit the phosphorylation of protein kinase B (Akt)/mammalian target of rapamycin (mTOR), reduce the expression of B cell lymphoma (Bcl-2), stimulate Bcl-2-associated X protein (Bax), and cleave Caspase 3 (activation), which then triggers apoptosis in breast cancer cells (Hu et al., 2018). In addition, curcumin induction also results in a decrease in the expression of antiapoptotic proteins such as Bcl-xl. The results of other studies indicate that curcumin can be used to combat drug resistance commonly found in various types of cancer, including breast cancer (Zoi et al., 2021). Table description: Values are the mean ± standard deviation from three replicates. Distinct superscript letters denote significant differences among groups (one-way ANOVA followed by Tukey's test, α = 0.05). Values that share the same letter are not statistically distinct.

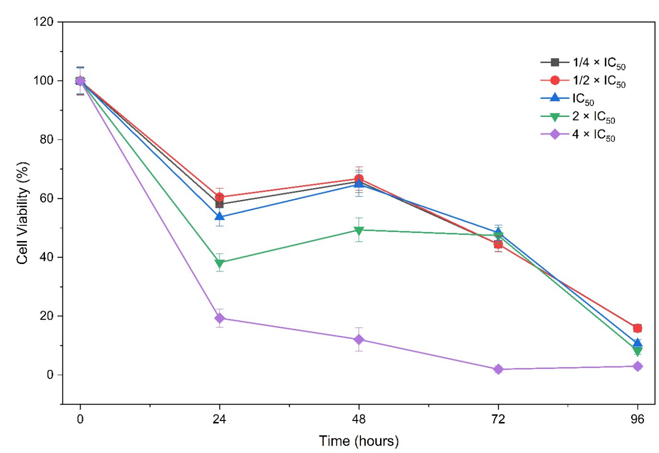

Figure 4. Percentage of T47D Cell Viability After Treatment with the Best Extract Combination at Incubation Times of 0, 24, 48, 72, and 96 Hours. A Two-Way ANOVA was Conducted, Followed by the Bonferroni Test for Statistical Analysis. Diverse Letters within the Same Time Group Signify a Substantial Difference

The total ion chromatogram results for soursop leaf extract are shown in Figure 3. The figure shows that the retention time ranges from 0 minutes to 17.5 minutes. At 11-14.5 minutes, the densest zone is found because there are many peaks with

Table 4. Results of Phytochemical Compound Identification from Turmeric Rhizome Extract Using LC/Q-TOF-MS

Name	Formula	RT (min)	Mass	CAS	Score	Score (DB)	Diff (ppm)
Affinin	$C_{14}H_{23}NO$	5.277	221.1781	25394-57-4	99.79	99.79	0.10
4-Ethylbenzaldehyde	$C_9H_{10}O$	6.077	134.0734	4748-78-1	95.05	95.05	0.25
9β-Hydroxyageraphorone	$C_{15}H_{24}O_2$	7.841	236.1778	97372-53-7	99.80	99.80	0.15
Terphenyllin	$C_{20}H_{18}O_5$	8.158	338.1153	52452-60-5	99.50	99.50	-0.10
TBBA/ 4-tert-Butylbenzoic acid	$C_{11}H_{14}O_2$	8.170	178.0994	98-73-7	99.98	99.98	0.03
a-a-Dimethylbenzenemethanol (a-Cumyl alcohol)	$C_9H_{12}O$	8.197	136.0889	617-94-7	87.85	87.85	0.07
Herniarin	$C_{10}H_8O_3$	8.278	176.0475	531-59-9	99.71	99.71	0.16
Curcumin	$C_{21}H_{20}O_6$	8.367	368.1259	458-37-7	97.28	99.51	-0.11
Terbuficin	$C_{30}H_{44}O_4$	9.159	468.3240	15534-92-6	99.80	99.80	0.08
1,3,5-Trimethylbenzene	C_9H_{12}	10.269	120.0941	108-67-8	94.34	94.34	0.25
3-Methylstyrene	C_9H_{10}	10.392	118.0785	100-80-1	96.18	96.18	0.23
Indene	C_9H_8	10.392	116.0628	95-13-6	87.70	87.70	0.20
Nonyphenol	$C_{15}H_{24}O$	10.891	220.1830	84852-15-3	99.65	99.65	0.25
1,2,4-Trimethylbenzene	C_9H_{12}	11.168	120.0938	95-63-6	87.89	87.89	-0.06
BHT-quinone methide (2,6-di-tert-butyl-4-methylene-2,5-cyclohexandienone)	$C_{15}H_{22}O$	11.193	218.1671	2607-52-5	99.95	99.95	0.07

high intensity. Many secondary metabolite compounds may be present at these peaks. The peaks decreased significantly after a retention time of 15 minutes, indicating low concentrations of non-polar compounds filling the chromatogram peaks. Table 5 shows that 15 candidate compounds have been identified from soursop leaf extract. LC/Q-TOF-MS results of soursop leaf extract showed that the majority of candidate compounds exhibited high database match scores, with a score >95, a score (DB) >95, and a difference between the measured mass and the database mass in the range of -0.11-0.25, indicating good, accurate mass matching under these analytical conditions.

The results of compound identification indicate the presence of a quercetin 3-rutinoside compound in soursop leaf extract. This compound has been recognised as both an antioxidant and an anticancer agent. Previous research results showed that the quercetin 3-rutinoside compound, at a concentration of 20 μM, exhibits toxic properties against MDA-MB-231 and MCF-7 breast cancer cells. Previous results also stated that quercetin 3-rutinoside induces cell cycle arrest in the G2/M and G0/G1 phases, which significantly encourages cell apoptosis. In addition, quercetin 3-rutinoside is also able to induce apoptosis by releasing cytochrome c from the mitochondria, which then binds to the Apaf-1 protein and triggers apoptosis (Ezzati et al., 2020).

3.3 Cytotoxic Activity of the Combination of Turmeric and Soursop Leaf Extracts Against T47D Cells

The results of the cytotoxicity assay on T47D cells using a combination of turmeric rhizome and soursop leaf extract are presented in Table 6. This IC_{50} value served as a reference and was analysed using the Design Expert application to determine the candidate combination according to the application. While the simplex lattice design is not a direct biological process, the optimization results illustrate the cellular response of T47D to the therapy combination. This optimization strategy facilitates the identification of interactions among components that may indicate potential interaction effects on T47D cells (Wang et al., 2025).

The results of the IC_{50} value with the combination ratio (Table 6) were analysed using Design Expert 13, showing a predicted R^2 value of 0.4024 and an adjusted R^2 of 0.5821. The difference in R^2 values is less than 0.2, indicating that the IC_{50} value obtained from the in vitro test is acceptable. Next is the analysis of the Adeq Precision value, which measures the ratio of the resulting data to the likelihood of data error (noise). The Adeq Precision value must be more than 4. Meanwhile, the Adeq Precision value obtained in this study was 5.354. Therefore, the resulting IC_{50} value can be used as a reference for further combination design (determining the three candidate combinations). The three candidate combinations recommended by the Design Expert application are presented in Table 7. The three candidate combinations were again subjected to cytotoxicity testing on T47D cells, and the IC_{50} values obtained are shown in Table 7. To assess the feasibility of interpreting the modeling results, the model fit parameters can be evaluated by analyzing the relatively low R^2 and adjusted R^2 values, indicating the model's ability to explain variations in IC_{50} responses. It is still limited. Although the Adeq Precision value is above the minimum threshold (>4), this parameter does not replace the need for strong goodness-of-fit. Thus, the model is not used for definitive optimum prediction but rather as an exploratory tool to select several ratios worthy of experimental retesting (Nouioura et al., 2023).

Following the in vitro assay on T47D cells, the three candidate combinations were identified. The combination ratio of turmeric rhizome extract to soursop leaf extract = 1:21, which had the lowest observed IC_{50} value. These results indicate that this combination ratio has the potential to enhance the growth-

Table 5. Results of Phytochemical Compound Identification from Soursop Leaf Extract Using LC/Q-TOF-MS

Name	Formula	RT (min)	Mass	Score	CAS	Score (DB)	Diff (ppm)
o-nitrotoluene	$C_7H_7NO_2$	0.568	137.0477	87.67	88-72-2	87.67	0.06
L-proline	$C_5H_9NO_2$	0.583	115.0633	91.55	147-85-3	87.64	-0.04
2,4-Dimethylquinoline	$C_{11}H_{11}N$	2.943	157.0891	87.71	1198-37-4	87.71	0.00
2-[4-(Diethylamino)-2-hydroxybenzoyl]benzoic acid	$C_{18}H_{19}NO_4$	3.104	313.1314	99.90	5809-23-4	99.90	-0.02
Norcodeine	$C_{17}H_{19}NO_3$	3.381	285.1368	99.49	467-15-2	99.49	0.31
Dimethylaminoethylbenzilate	$C_{18}H_{21}NO_3$	3.427	299.1524	99.75	968-46-7	99.75	0.23
Cinnamolaurine	$C_{18}H_{19}NO_3$	3.451	297.1365	85.66	25866-03-9	85.66	0.03
Corytuberine	$C_{19}H_{21}NO_4$	3.497	327.1470	99.96	517-56-6	99.96	-0.01
Cinnamoylcocaine	$C_{19}H_{23}NO_4$	3.693	329.1628	99.94	521-67-5	99.94	0.05
Quercetin 3-rutinoside	$C_{27}H_{30}O_{16}$	4.145	610.1537	99.69		99.69	0.27
Kaempferol 3-glucoside-7rhamnoside	$C_{27}H_{30}O_{15}$	4.368	594.1587	99.82	2392-95-2	99.82	0.21
Dihydrothebaine	$C_{19}H_{23}NO_3$	4.712	313.1679	99.94	561-25-1	99.94	0.06
Apocodeine	$C_{18}H_{19}NO_2$	4.953	281.1415	99.84	641-36-1	99.84	-0.03
Indobufen	$C_{18}H_{17}NO_3$	5.169	295.1211	99.67	63610-08-2	99.67	0.24
2-cyclohexylphenol	$C_{12}H_{16}O$	5.289	176.1203	99.83	119-42-6	99.83	0.15

Table 6. Results of the Cytotoxic Test of the Combination of Turmeric Rhizome Extract and Soursop Leaf Extract on T47D Cells

Combination type	Combination Ratio		IC_{50} (μg/mL)
	Turmeric rhizome extract	Soursop leaf extract	
1	1	0	39.4 ± 1.0^a
2	1	1	37.7 ± 1.4^a
3	0	1	32.6 ± 5.6^a
4	1	0	39.7 ± 1.3^a
5	1	3	35.6 ± 1.2^a
6	3	1	34.5 ± 4.2^a

Table description: Values are the mean ± standard deviation from three replicates. Distinct superscript letters denote significant differences among groups (one-way ANOVA followed by Tukey's test, α = 0.05). Values that share the same letter are not statistically distinct.

inhibitory activity of T47D cells. This ratio is regarded as the most promising choice due to its lowest measured IC_{50} for further assessment. The candidate combination is also used to determine the selectivity index (IS). A higher IS value indicates better selectivity. The selectivity index value is measured by comparing the IC_{50} value of the best combination on T47D cells with the IC_{50} value on normal Vero cells. The cytotoxicity assay results for the candidate combination with Vero cells produced an IC_{50} value of 75.9 ± 9.1 μg/mL, reflecting an IS value of 2.4 for the candidate combination. Other research suggests that an IS value ≥1 indicates the extract's potential anticancer activity due to its safety against normal cells (Klimek et al., 2021). Furthermore, the standard IS value used is greater than 2, indicating better selectivity (Rashidi et al., 2017). Therefore, the candidate combination extract exhibited cytotoxic activity toward T47D cells, with lower effects observed in normal cells under the tested conditions.

Previous research results showed that turmeric ethanol extract has cytotoxic activity against murine leukemia P-388 cells (IC_{50} = 6.15 μg/mL) (Wati et al., 2022), MCF7 cells (IC_{50} = 26.30 μg/mL), T47D cells (IC_{50} = 17.2 ± 3.2 μg/mL), HepG2 cells (IC_{50} = 105.6 ± 8.6 μg/mL), HeLa cells (IC_{50} = 54.4 ± 1.5 μg/mL), and WiDr cells (IC_{50} = 81.3 ± 2.4 μg/mL) (Haryanti et al., 2025). Turmeric extract exhibits variable cytotoxicity, as indicated by varying IC_{50} values. The cytotoxic activity of turmeric extract is also influenced by the type of test cell line. In this study, turmeric extract exhibited cytotoxicity against T47D cells, with an IC_{50} of 39.55 μg/mL. Meanwhile, previous research showed that curcumin (a single compound) exhibited cytotoxicity against T47D cells, with an IC_{50} of 19.67 μM (Nasiri et al., 2013). Meanwhile, previous research showed that the ethanol extract of soursop leaves exhibited cytotoxicity against T47D cells with an IC_{50} of 109.91 ± 3.04 μg/mL (Fertilita et al., 2020). In this study, soursop leaf extract showed cytotoxicity against T47D cells, with an IC_{50} value of 32.6 ± 5.6 μg/mL.

Table 7. Cytotoxicity Assay Results of the Three Candidate Combinations of Turmeric Rhizome and Soursop Leaf Extracts on T47D Cells

Three candidate combination types	Combination ratio		IC_{50} prediction by design expert 13 (μg/ml)	IC_{50} values of the in vitro test (μg/ml)
	Turmeric rhizome extract	Soursop leaf extract		
1	1	39	33.4	33.2 ± 0.8^a
2	1	21	33.5	32.2 ± 2.5^a
3	1	10	33.8	33.8 ± 0.6^a

Table description: Values represent the mean ± standard deviation derived from three replicates. Unique superscript letters indicate statistically significant differences between groups (one-way ANOVA with subsequent Tukey's test, $\alpha = 0.05$). Values that share the same letter are not statistically distinct.

Table 8. Results of Apoptosis Induction Analysis of the Candidate Combination of Extracts on T47D Cells

Treatment	% T47D Cell Distribution		
	Necrosis	Apoptosis	Living cell
Cell control	0.42 ± 0.01^a	4.12 ± 0.13^a	95.51 ± 0.20^a
Doxorubicin	41.83 ± 0.32^b	47.12 ± 0.12^b	11.12 ± 0.41^b
½ × IC_{50}	3.04 ± 0.12^c	15.44 ± 0.32^c	81.63 ± 0.21^c
IC_{50}	46.82 ± 0.22^d	18.22 ± 0.94^d	35.24 ± 0.62^d
2 × IC_{50}	5.51 ± 0.61^e	93.83 ± 0.74^e	0.74 ± 0.12^e

Table description: Values represent the mean ± standard deviation derived from three replicates. Statistical analysis was performed in each column using one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$). The same letters in the same column are not significantly different.

3.4 Antiproliferative Activity of the Candidate Extract Combination Against T47D Cells

The candidate combination of turmeric rhizome and soursop leaf extracts (in a 1:21 ratio) showed the lowest observed IC_{50} value of 32.2 ± 2.5 ±g/mL. This candidate combination was used for further testing, including the antiproliferation assay on T47D cells. The cell proliferation assay was conducted to observe the inhibition of the proliferation pattern of T47D cells at incubation times of 0, 24, 48, 72, and 96 hours. The decrease in T47D cell viability after administration of the optimal combination extract is shown in Figure 4.

The results showed that the longer the incubation time, the lower the average viability of T47D cells. Furthermore, the candidate combination extract decreased cell viability as the concentration of the extract increased. This indicates that the candidate combination extract significantly inhibited T47D cell proliferation, particularly at concentrations of 1, 2, and 4 × IC_{50}. A two-way ANOVA showed that treatment and time each significantly affected cell viability ($p < 0.001$), and there was a significant interaction between treatment and time ($p < 0.001$). This indicates that the treatment effect on cell viability depends on exposure time. A Bonferroni test for simple effects indicated that at 0 hours, there was no significant difference in viability across the doses. High doses (particularly 2 × IC_{50} and 4 × IC_{50}) significantly decreased viability within 24–48 hours compared to low–medium levels. At 72 hours, 4 × IC_{50} showed markedly reduced viability compared to other dosages. At 96 hours, the disparity between groups diminished due to low survivability across all groups; however, the 4 × IC_{50} remained much lower than other dosages (¼, ½, and 1 × IC_{50}). The turmeric rhizome and soursop leaves, components of the combined extract, exhibit antiproliferative effects due to the presence of several compounds, including curcumin and quercetin 3-rutinoside. Curcumin decreased p53 and ERα expression at concentrations of 5-60 μM. Low p53 and ERα expression inhibited cancer cell proliferation (Guo et al., 2022). Quercetin 3-rutinoside has also been shown to increase caspase-3 protein expression and decrease p53 expres-

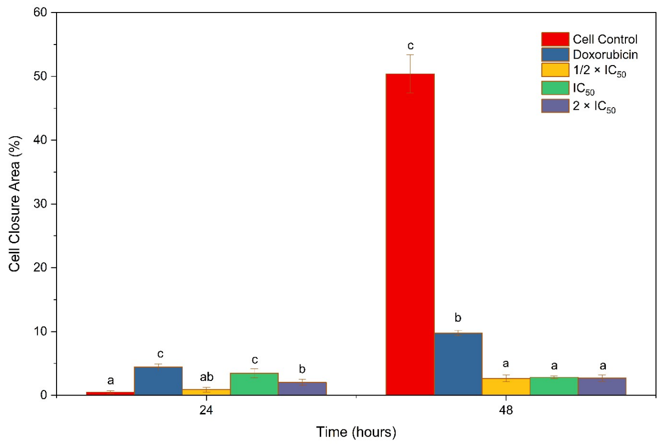

Figure 5. Percentage Area of T47D Cells Treated with the Candidate Combination Extract. Statistical Analysis was Performed Using a Two-Way ANOVA Followed by the Bonferroni Test. Different Letters at the Same Time Indicate Significant Differences

sion, thereby triggering apoptosis in cancer cells and inhibiting their proliferation (Pandey et al., 2021).

3.5 Antimetastatic Activity of the Candidate Combination Extract Against T47D Cells

The candidate combination extract of turmeric rhizome and soursop leaves was tested for antimetastatic activity against T47D cells. The results showed that the candidate combination extract was able to inhibit metastasis in T47D cells. Antimetastatic activity was indicated by a slower rate of wound closure area over time compared to a control group. The percentage of T47D cell coverage area is shown in Figure 5. The study results of a two-way ANOVA indicated a significant interaction between treatment and time regarding closure area (metastasis activity) in T47D cells. At 24 hours post-scratch, the % closure area in the control group did not significantly differ from that of the ½ × IC_{50} group (Bonferroni's follow-up test, p=0.440). The control group exhibited markedly different area closure rates compared with doxorubicin, IC_{50}, and 2 × IC_{50} ($p < 0.001$). Moreover, the area closure in the doxorubicin group did not differ substantially from the IC_{50} (p=0.629). At 48 hours post-scratch, the ½ × IC_{50} group showed no significant difference compared with the IC_{50} and 2 × IC_{50} group (Bonferroni's post hoc test, p=1.000). Conversely, all other treatment comparisons showed a significant difference (most $p < 0.001$), indicating that each treatment produced varying area closure at 48 hours.

The results of the study showed that T47D cell metastasis was actively carried out at 48 hours of incubation. This can be observed from the data of control cells incubated for 24 hours, which did not show metastasis; however, the control cells exhibited metastasis after 48 hours of incubation (Figure 5). The metastatic ability of each cell is greatly influenced by the expression of matrix metalloproteinases (MMPs). Cancer cells that express high levels of MMP will be invasive and metastatic (Bartsch et al., 2003; Su et al., 2008). The results of previous studies showed that the area of closure that occurred in T47D cells at 24 and 48 hours of incubation was 14.96 ± 7.19% and 59.34 ± 11.93%, respectively. These data indicate that T47D cells metastasize rapidly at 48 hours of incubation (To'bungan et al., 2022). In this study, significant inhibition of metastatic activity at 48 h was observed in the ½ × IC_{50}, IC_{50}, and 2 × IC_{50} treatment groups, with no significant differences among these concentrations, resulting in closure areas of 2.64 ± 0.52%, 2.81 ± 0.20%, and 2.71 ± 0.51%, respectively. Moreover, all three concentrations exhibited approximately 48% greater inhibition compared with the control group. These concentrations demonstrated significantly stronger antimetastatic effects than doxorubicin treatment (Figure 5).

3.6 Apoptosis Induction of the Candidate Extract Combination Against T47D Cells

The candidate combination extract of turmeric rhizome and soursop leaves was tested for its ability to induce apoptosis in T47D cells. The results of the apoptosis induction test for the selected candidate combination extract are shown in Figure 6. The results showed that administration of the candidate combination extract can induce apoptosis along with increasing concentrations given. The percentage of apoptosis induced by the selected candidate combination extract is presented in Table 8.

The increase in cell apoptosis following the administration of a combination of turmeric rhizome and soursop leaf extracts may be due to the presence of phytochemical compounds in the extract combination. LC/Q-TOF-MS results indicate that curcumin was identified in the turmeric rhizome extract. Curcumin reduces cyclooxygenase-2 (COX-2) expression, which ultimately prevents inflammation and inhibits the growth and metastasis of cancer cells. Curcumin inhibits nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), thereby suppressing the expression of anti-apoptotic genes and increasing the expression of apoptotic genes (Ebnudesita et al., 2022). Previous research has shown that *Annona muricata* leaf extract induces apoptosis in A549 cells, with induction rates of 34.1% (early apoptosis) and 42.6% (late apoptosis) after treatment at 10 μg/mL for 72 hours. The study explained that soursop leaf extract significantly increased ROS formation and decreased MMP through mechanisms such as increased Bax, decreased Bcl-2, and the release of cytochrome c into the cytosol. Cytochrome c release triggers Caspase 9 activation, which subsequently activates Caspase 3, thereby inducing apoptosis. Furthermore, soursop leaf extract also suppressed NF-κB-induced translocation from the cytoplasm to the nucleus (Moghadamtousi et al., 2014).

Table 9. Results of Cell Cycle Arrest Analysis of the Candidate Combination Extract on T47D Cells

Treatment	% Distribution of T47D Cells in various phases			
	G1	S	G2/M	Sub-G1
Cell control	48.12 ± 4.41^{bc}	16.31 ± 1.62^{a}	34.93 ± 2.32^{a}	0.62 ± 0.63^{a}
Doxorubicin	34.31 ± 1.02^{ab}	18.02 ± 0.13^{a}	44.91 ± 0.82^{a}	2.81 ± 0.13^{a}
½ × IC_{50}	53.81 ± 0.53^{c}	14.82 ± 0.33^{a}	30.21 ± 0.22^{a}	1.22 ± 0.04^{a}
IC_{50}	27.61 ± 7.52^{a}	20.32 ± 4.54^{a}	45.22 ± 9.53^{a}	6.52 ± 7.01^{a}
2 × IC_{50}	34.51 ± 1.52^{ab}	13.91 ± 2.92^{a}	44.41 ± 4.22^{a}	5.22 ± 5.41^{a}

Table description: Values are presented as mean ± SD (n = 3 independent experiments). Statistical analysis was conducted for each column utilizing one-way ANOVA, followed by Tukey's post-hoc test (α = 0.05). The identical letters inside the same column exhibit no significant difference.

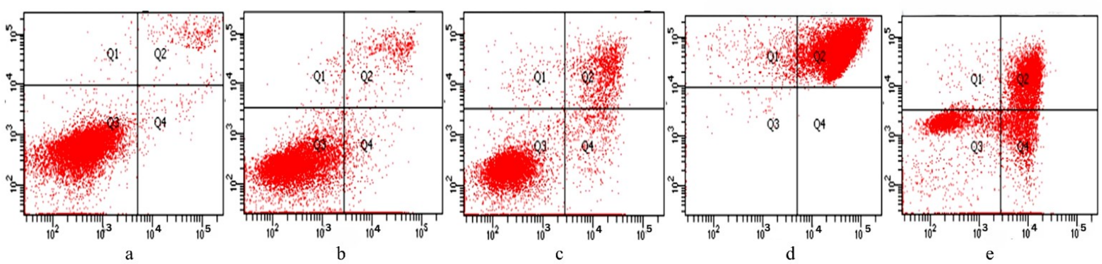

Figure 6. Results of T47D Cell Apoptosis Analysis Using Flow Cytometry After Treatment with Extracts at Various Concentrations. The Cell Distribution Shows the Distribution in Each Quadrant: Q1 (Necrotic Cells), Q2 (Late Apoptotic Cells), Q3 (Live Cells), and Q4 (Early Apoptotic Cells). Figure Captions: a: Analysis of Apoptosis of T47D Cells as Control Cells, b: Analysis of Apoptosis of T47D Cells After Treatment with ½ × IC_{50} of the Candidate Combination Extract, c: Analysis of Apoptosis of T47D Cells After Treatment with the IC_{50} of the Candidate Combination Extract, d: Analysis of Apoptosis of T47D Cells After Treatment with 2 × IC_{50} of the Candidate Combination Extract, e: Analysis of Apoptosis of T47D Cells After Doxorubicin Treatment

3.7 Cell Cycle Arrest by the Candidate Combination of Extracts Against T47D Cells

The increase in apoptosis induction in T47D cells due to the candidate combination extract treatment was further investigated by examining cell cycle inhibition in each phase. The results of the T47D cell cycle inhibition analysis, conducted using flow cytometry after administering the candidate combination extract, are presented in Figure 7. Administration of the candidate combination extract at IC_{50} and 2 × IC_{50} doses can inhibit cell cycle progression in the G2/M phase. The percentage of cell cycle inhibition in each phase is shown in Table 9.

T47D cell cycle inhibition by the candidate combination of turmeric rhizome and soursop leaves may result from active compounds with potential anticancer properties. Turmeric rhizome has been identified as a source of curcumin compounds with potential anticancer activity. Previous research results indicate that curcumin can modulate p53 gene expression. The p53 gene plays a crucial role in regulating the cell cycle by activating the checkpoint mechanism, thereby inhibiting the proliferation of cancer cells by arresting the cell cycle in the G2/M phase (Astuti et al., 2012). Soursop leaf extract is known to contain quercetin 3-rutinoside compounds. Quercetin 3-rutinoside compounds have been known to have anticancer potential. Previous research results have shown that quercetin 3-rutinoside is known to arrest the G2/M phase of the cell cycle at a dose of 20 μM in MDA-MB-231 breast cancer cells. Quercetin 3-rutinoside at a dose of 50 μM can inhibit the G0/G1 phase cell cycle in MCF-7 cells (Iriti et al., 2017). The candidate combination of turmeric rhizome extract and soursop leaf extract (1:21) is a promising combination. This combination exhibited the lowest recorded IC_{50} in cytotoxicity

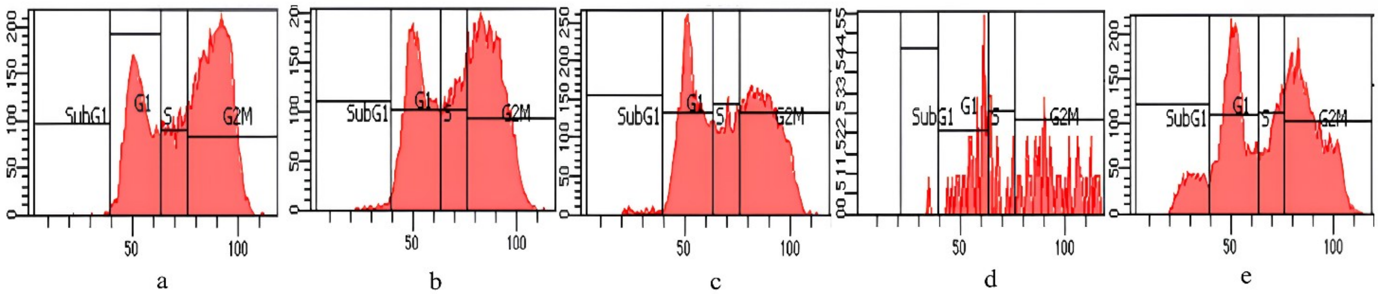

Figure 7. The Results of the Cell Cycle Arrest Analysis of T47D Cells Using Flow Cytometry Following Treatment with the Candidate Combinations of Extracts at Various Concentrations. Image Description; a: Analysis of T47D Cell Cycle Inhibition as Control Cells, b: Analysis of T47D Cell Cycle Inhibition After Treatment with ½ × IC_{50} of the Candidate Combination Extract, c: Analysis of T47D Cell Cycle Inhibition After Treatment with IC_{50} of the Candidate Combination Extract, d: Analysis of T47D Cell Cycle Inhibition After Treatment with 2 × IC_{50} of the Best Combination Extract, e: Analysis of T47D Cell Cycle Inhibition After Doxorubicin Treatment

assays against T47D cells. Under the evaluated conditions, it reduced cell viability and migration, correlating with heightened apoptotic cell populations and G2/M phase accumulation in cell-cycle analysis.

4. CONCLUSIONS

A qualitative phytochemical profile putatively identified 15 compounds in the turmeric extract. One of the compounds found was curcumin, which has been widely documented to have anticancer properties. A qualitative phytochemical analysis tentatively identified 15 compounds in the soursop leaf extract, including quercetin-3-rutinoside, which is well documented for its anticancer properties. The most promising combination ratio of turmeric rhizome and soursop leaf extracts was determined to be 1:21, exhibiting an IC_{50} value of 32.2 ± 2.5 μg/mL against T47D breast cancer cells. This combination demonstrated antiproliferative activity and reduced T47D cell migration *in vitro*. The combination was associated with elevated apoptotic cell populations and G2/M phase accumulation in cell cycle analyses.

5. ACKNOWLEDGEMENT

The authors gratefully acknowledge the research grant funding from the National Agency for Research and Development (BRIN) Health Organization Program House for the 2023 fiscal year, under Decree No. 4/III.9/HK/2023. They also gratefully acknowledge the facilities, scientific, and technical support provided by the BRIN Tawangmangu Drug and Traditional Medicine Raw Materials Laboratory. The authors acknowledge the facilities and scientific and technical support from the Advanced Chemical Characterization Laboratory, National Research and Innovation Institute, through E-Science Services-BRIN.

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