Anticancer potential of turmeric (*Curcuma longa*) ethanol extract and prediction of its mechanism through the Akt1 pathway [version 1; peer review: awaiting peer review]

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Abstract

**Background:** Turmeric (*Curcuma longa*) has high potential as a traditional anticancer drug. This study aimed to analyze the anticancer activity of turmeric ethanol extract on T47D cells and examine the interaction of Akt1 protein with compounds contained in turmeric.

**Methods:** The cytotoxicity assay was conducted using WST-1 reagents. Apoptosis assay used annexin V-PI, whereas cell cycle assay used PI, and then the results were analyzed using a flow cytometer. LC-HRMS analysis was conducted to identify the active compounds. Docking between Akt1 and ligands was performed using Autodock 4.2 software. Molecular dynamics simulations were conducted using YASARA with a time parameter of 20 ns, pH 7.4, and 37°C.

**Results:** The extract had a strong toxicity on T47D cells (cytotoxicity IC₅₀ value: 26.36 ± 1.55 µg/mL). The extract induced apoptosis of T47D cells at the IC₅₀ dose (~30% cells) and induced the cell cycle arrest in G1 phase. Curcumin, 2-hydroxycinnamic acid and caryophyllene oxide had lower binding energy into Akt1 than AZD5363 used as a positive control. Curcumin, Ar-turmerone, and α-curtcumene bind in the ATP binding pocket of Akt1, so the compounds have a high potential to be an ATP-competitive Akt1 inhibitors. The interaction of Akt1 with the compound contained in turmeric had an RMSD backbone value that was more stable than that of ATP and AZD5363. Root-mean-square fluctuation values indicated that amino acid residues that had an essential role in ligand binding sites were stable during simulation.

**Conclusions:** The turmeric ethanol extract had a potential anti-cancer effect by inducing apoptosis and inhibiting cell cycle progression on T47D cells. The docking analysis showed that the active compounds of the extract, such as curcumin, Ar-turmerone, caryophyllene oxide, and α-curtcumene, were able to bind into the ATP binding pocket of Akt1 that might inhibit the protein activity and induce cell cycle arrest.
Keywords
Akt1, apoptosis, docking, cell cycle, molecular dynamics, T47D

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Introduction
In 2015, cancer was the number one cause of death globally before the age of 70 years in 91 countries. In 2012, breast cancer was the most common type of cancer among women in Asia-Pacific region and the fourth most common cause of cancer-related deaths. An increase in breast cancer mortality rates has been seen in Malaysia, Thailand, Hong Kong, and Singapore. Mortality caused by breast cancer was higher in women younger than 50 years than in women older than 50 years. The risk of developing breast cancer is influenced by several aspects such as heredity, ethnicity, contraception, alcohol consumption, obesity, not being able to breastfeed, smoking, and lack of physical activity.

The most common treatments used for breast cancer are chemotherapy and radiotherapy. The side effects of chemotherapy and radiotherapy in patients are peripheral neuropathy, nausea, vomiting, fatigue, alopecia (loss of hair), diarrhea, and constipation, and the most dangerous is febrile neutropenia. Moreover, those treatments are relatively expensive; hence, using traditional herbal medicine is the other option. Turmeric (Curcuma longa) is a medicinal plant traditionally used to treat various diseases such as eye diseases, smallpox, digestive disorders, liver disorders, and itching. It is known to be antiparasitic, anti-infectious, antiperiodic, astringent, diuretic, and tonic. Eating foods containing turmeric regularly can reduce the risk of developing various diseases such as rheumatism, heart disease, tumors, cancer, Alzheimer’s disease, and other infectious diseases.

The active compounds of C. longa have antioxidant activity, stabilizing damaged cells by supplying the electrons for free radicals. A 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay is one method to evaluate the radical scavenging activity of antioxidants that is accurate, easy, and economical. The DPPH, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to a colorless (violet to yellow) compound.

Reportedly, turmeric ethanol has a moderate toxicity on HeLa cells (cervical cancer cell line). One of the active compounds in turmeric is curcumin that is present in abundance in rhizome (70%-80%). Curcumin has antiproliferative functions in some cancer cells and also acts as an inhibitor of nuclear factor kappa B (NF-kB) transcription factors, B-cell lymphoma 2 (Bcl-2), cyclooxygenase-2 (COX-2), tumor necrosis factor alpha (TNF-α), and matrix metalloproteinase-9 (MMP-9). In addition, curcumin can affect various growth factor receptors and cell adhesion molecules involved in tumor growth, angiogenesis, and metastasis. Previous research stated that curcumin could potentially inhibit the activity of protein kinases A and C. Thus, curcumin might also inhibit the activity of protein kinase B (Akt1), which has an essential role in cell growth and proliferation.

Akt1 comprises 488 amino acids (56.7 kDa) and inhibits apoptosis through the inactivation of proapoptotic proteins such as Bad (Bcl-2 antagonist cell death) and MDM2 (mouse double minute-2 homologs), causing p53 degradation. The Akt1 protein has been found to be overexpressed in breast cancer cells either ER+ or HER+. Therefore, Akt1 is often used as the main target in cancer therapy and prevention. Some Akt1 inhibitor compounds have been developed to target adenosine triphosphate (ATP) binding pocket, which is called ATP-competitive Akt1 inhibitor. This inhibitor competes with ATP to bind to the ATP binding pocket so Akt1 cannot phosphorylate the native substrate. Hence, this study aimed to analyze the antioxidative, cytotoxic, apoptotic effects, and cell cycle arrest of turmeric ethanol extract on breast cancer (T47D) cells and investigate the toxicity mechanism by using in silico analysis.

Methods
Sample extraction
Turmeric powder was obtained from Materia Medika, Batu, and was then extracted with 96% ethanol (Merck) at a ratio of 1:10 (w/v) using the maceration method for 24 h and solvent replacement was done four times at room temperature. Macerate was filtered with filter paper No. 41 (Whatman). The filtrate was evaporated using a rotary evaporator (Buchi) at a temperature of 50°C and the run time was 30 min. A full scan was done at 70,000 resolution and data-dependent MS2 was conducted at 17,500 resolution. Data was processed using Compound Discoverer version 3.2 (Thermo Scientific) with an mzCloud MS/MS Library.

Liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis
LC-HRMS analysis was conducted at Central Laboratory of Life Sciences, Brawijaya University. The ethanol extract of turmeric was dissolved in dimethyl sulfoxide (DMSO) (Merck) to make a final volume of 1300 μL. Further, it was spun down at 6000 rpm for 2 min. The supernatant was taken and filtered using a 0.22 μm syringe filter and put into the vial. The sample was ready to be put into the autosampler and then injected into the LC-HRMS (Thermo Scientific Dionex Ultimate 3000 RSLCnano with microflow meter). The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The chromatographic separation was performed by analytical column Hypersil GOLD aQ 50 x 1 mm x 1.9 μm particle size with an analytical flow rate of 40 μL/min. The column temperature was set at 40°C and the run time was 30 min. A full scan was done at 70,000 resolution and data-dependent MS2 was conducted at 17,500 resolution. Data was processed using Compound Discoverer version 3.2 (Thermo Scientific) with an mzCloud MS/MS Library.
DPPH scavenging assay
Turmeric ethanol extract and standard solution (ascorbic acid) (Sigma-Aldrich) were diluted in 96-well plates to obtain concentrations of 250, 125, 62.5, 31.25, 15.625, 0 μg/mL in 100 μL volume; 100 μL of 0.4 mM DPPH (Sigma-Aldrich) was added to each well. The plates were incubated in a thermos mixer at room temperature for 30 min. The absorbance was measured at λ490 nm using ELx808™ Absorbance Microplate Reader (BioTek Instrument). The absorbance data were imputed into the % inhibition formula to create a linear regression curve. The value 50 was interpolated into the linear regression equation instead of “y”. Therefore, the IC₅₀ value of each sample or standard was obtained.

\[
\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance sample or standard}}{\text{Absorbance of control}} \times 100
\]

Cell culture preparation
Breast cancer cell line T47D was obtained from the Laboratory of Animal Physiology, Structure, and Growth, Brawijaya University. The cell was cultured in complete medium (RPMI 1640 (Gibco) + 10% Fetal bovine serum (FBS) (Gibco) + 1% Penicillin–Streptomycin (Gibco)). The cells were stored at 37°C in a 5% CO₂ incubator (Esco) until reaching an 80% confluence.

Cytotoxicity assay
The T47D human breast cancer cell line was seeded into a 96-well plate at a density of 5000 cells per well and incubated overnight. The cells were treated with various concentrations of turmeric ethanol extract (0, 5, 10, 20, and 40 μg/mL) and DMSO (Merck) (0.5%) as a control followed by incubation at 37°C in a 5% CO₂ incubator for 24 h. Treatment media was replaced with media containing 5% Water-soluble tetrazolium salts (WST-1) (Sigma-Aldrich) and incubated in the dark for 30 min. Absorbance was measured using ELx808™ Absorbance Microplate Reader (BioTek Instrument) at 450 nm. The IC₅₀ was determined by an inhibition curve.

Apoptosis assay
The T47D cell line was seeded into a 24-well plate at a density of 50,000 cells per well and incubated (37°C, CO₂ 5% incubator) overnight. The cell was treated with 0 (untreated), 13.2, 26.4, and 52.77 μg/mL turmeric ethanol extract and incubated at 37°C in a 5% CO₂ incubator for 24 h. Cells were harvested and Annexin V and propidium iodide (PI) (BioLegend) was added then incubated in the dark for 20 min. The analysis was conducted using the FACSCalibur analyzer (BD bioscience), and the data were analyzed using CellQuest software version 2.0 (RRID:SCR_014489) (An open-access alternative is Flowing Software 2.5.1 (RRID:SCR_015781)).

Cell cycle assay
The T47D cell line was seeded at a density of 75,000 cells per well on a 24-well plate and incubated at 37°C in a 5% CO₂ incubator until confluent. Then, the cells were treated with 0 (untreated), 13.2, 26.4, and 52.7 μg/mL turmeric ethanol extract and incubated at 37°C in a 5% CO₂ incubator for 24 h. The cells were collected and resuspended with PI and RNAs (Abcam) and incubate at 37°C for 30 min. The analysis was conducted using the FACSCalibur analyzer (BD bioscience) and analyzing data using CellQuest software.

Statistical analysis
Statistical analysis of cytotoxicity, DPPH, apoptosis and cell cycle assays were done using One-Way Analysis of Variance (ANOVA) with Tukey honest significant difference (HSD) method when comparing five groups. p < 0.01 and p < 0.001 was used to determine a statistically significant difference. The error bars on graphs represent ± standard deviation (SD) of three repetitions. The analysis was performed using SPSS version 23 (SPSS, Inc., Chicago, IL, USA) (RRID:SCR_016479). The raw data of these analyses are provided as underlying data.²³

Docking
The three-dimensional structure of the Akt1 protein (PDB ID: 6HHF) as a receptor was obtained from the RCSB database. The 3-D structures of the compound such as curcumin (CID: 969516), ferulic acid (CID: 445858), α-curcumen (CID: 92139), Ar-turmerone (CID: 558221), corophyllene oxide (CID: 1742210), p-cymene (CID: 7463), 2-hydroxycinnamic acid (637540), ATP (CID: 5957) as a native ligand, and AZD5363 (CID: 25227436) as a positive control were obtained from PubChem database. Akt1 protein preparation was performed using the BIOVIA Discovery Studio 2019 software (Dassault Systèmes Biovia, San Diego, California, USA) (RRID:SCR_015651), and ligands were performed with PyRx 0.8 software (RRID:SCR_018548). Each ligand (curcumin, ATP, and AZD5363) was docked one by one with the Akt1 protein with Autodock 4.2 software (RRID:SCR_012746) integrated into PyRx 0.8 using the Lamarckian genetic algorithm method with Grid Center X: 8.7122, Y: 4.2212, and Z: 11.334 and Grid Point X: 173, Y: 130, and Z: 152. Docking results were visualized using the BIOVIA Discovery Studio 2019 software. The raw data of these analyses are provided as underlying data.²³
Molecular dynamic simulation

Molecular dynamics simulation was performed using Yet Another Scientific Artificial Reality Application (YASARA) structure software version 20.12.24 (RRID:SCR_017591) with a running time of 20,000 ps and autosave every 25 ps (An open-access alternative is GROMACS 2022.2 (RRID:SCR_014565). The parameters used were the following cell physiological conditions: temperature of 37°C, pH 7.4, salt content of 0.9%, and pressure of 1 atm. The simulation was conducted using the macro md_rundfast program. Analysis of root-mean-square deviation (RMSD) backbone protein was conducted using macro md_analyzer, and root-mean-square fluctuation (RMSF) analysis was performed using the macro md_analyzeres.23

Results

DPPH scavenging activity of the extract

DPPH assay is an antioxidant assay method, which has been quite popular in recent years because it is simple and highly sensitive. The antioxidant activity is proportional to the loss of DPPH because DPPH accepts hydrogen from antioxidant compounds.24 Figure 1A shows the results of the DPPH scavenging activity of turmeric ethanol extract. The IC50 value was calculated to determine the sample concentration required for the 50% inhibit radicals. The lower the IC50 value, the higher the antioxidant activity of the sample.27,28 The results of the DPPH activity test showed that the IC50 value of the turmeric ethanol extract was 211.73 ± 2.52 ppm. According to Ref. 29, an extract that possesses IC50 values ranging from 100 to 250 ppm is considered to have weak antioxidant activity. Hence, the IC50 value of turmeric ethanol extract was included in the weak antioxidant activity category. The LC-HRMS results obtained compounds that have antioxidant activity, such as curcumin,30 ferulic acid,31,32 caryophyllene oxide,33 p-cymene,34 and 2-hydroxycinnamic acid35 (Table 1). Cancer cells produce more ROS than normal cells, but cancer cells adapt by increasing the production of enzymes and antioxidant molecules to survive.36 Therefore, constituents with high antioxidant activity will support the growth of cancer cells.37 Turmeric ethanol extract has weak antioxidant activity, so it is unlikely to support the growth of cancer cells.

Turmeric ethanol extract inhibited the proliferation of the T47D cell line

The toxicity test aimed to determine the toxic effect of the turmeric ethanol extract on T47D cells. This study shows that turmeric ethanol extract has toxic effects on T47D cells, which have ER + and PR + characteristics.25 The results showed that the proliferation of the T47D cell line was dose-dependently inhibited by turmeric ethanol extract (Figure 1B), with the IC50 value being 26.36 ± 1.55 μg/mL. Cytotoxic activity was divided into three based on IC50 values, IC50 < 100 μg/mL was a potential cytotoxic, IC50 < 1000 μg/mL was moderate cytotoxic, and IC50 > 1000 μg/mL had no cytotoxic activity. As per the National Cancer Institute for crude extracts of a natural plant with IC50 values < 30 μg/mL is considered to have a potential cytotoxicity effect.38 The toxic effect of turmeric ethanol extract is predicted due to the active compound content. Compounds with potential cytotoxic characteristics could be used as anticancer agents, whereas moderate cytotoxic compounds could prevent or inhibit cancer cells or for chemoprevention.40 Therefore, turmeric ethanol extract was included as a potential cytotoxic agent that could potentially be used as an anticancer agent.

Turmeric ethanol extract promoted the apoptosis of the T47D cell line

Turmeric ethanol extract induces T47D apoptosis in a dose-dependent manner (Figure 1C and D). The dose used was based on the result of IC50 of cytotoxic assay. They were ½ × IC50, IC50, 2 × IC50 (i.e., 13.2, 26.4, and 52.7 μg/mL). The results showed that the treatment with turmeric extract at a concentration of 26.4–52.7 μg/mL had shown an apoptosis induction activity of around 30–90%. These data correspond with previous research that curcumin in turmeric ethanol extract induces apoptosis11 by increasing pro-apoptotic proteins BAX and caspase 3 while decreasing the expression of anti-apoptotic proteins such as Bcl-2.41 Curcumin also has selective killing of cancer cells and does not affect normal cells. Furthermore, curcumin increased the proliferation of T cells, CD4+ cells, and B cells and increased the IL-12 production, which is harmless to the immune system.32 Ar-turmerone in turmeric caused DNA fragmentation in various leukemia cancer cell lines.43 2-Hydroxycinnamic acid stimulated apoptosis through increased caspase-3 activity in some human pancreatic cancer cell lines.44 α-Curcumen induced apoptosis by releasing cytochrome c from mitochondria in the cervix cancer cell line.45

Turmeric ethanol extract induced cell cycle arrest in the G1 phase

Based on the cell cycle assay results, it could be concluded that turmeric ethanol extract induced T47D cell cycle arrest in G1 phases (Figure 1E). Cell accumulation in the G0/G1 phase increased along with extract concentrations. Cell cycle arrest in the G1 phase also could be induced by inhibiting the activity of the protein Akt1. Akt1 had an essential role in the G1/S transition. Therefore, inhibition of Akt1 activity led to cell arrest at G0/G1.36 Our data correspond to the previous research that turmeric curcumin inhibits the MCF-7 breast cancer cell cycle in the G0/G1 phase.37 Turmeric extract also inhibited the proliferation and induced the apoptosis of breast cancer cuboid epithelial cells in an animal model.38
Figure 1. Turmeric ethanol extract had antioxidant activity and induced cell toxicity, apoptosis, and cell cycle arrest to the T47D cell line. (A) The antioxidant property of extract. (B) Cell toxicity effect of the extract (24 h). (C & D) Apoptosis induction by the extract (24 h). (E) Cell cycle arrest induction by the extract (24 h). The data represent the mean of three repetitions. The number of (*) indicates the level of significant difference with the control (*p < 0.01 and **p < 0.001).

Table 1. Compounds obtained from the results of the LC-HRMS analysis.

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<th>Name</th>
<th>Formula</th>
<th>mz Cloud best match</th>
<th>Concentration (%)</th>
<th>Molecular structure</th>
<th>Anticancer reference</th>
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<tr>
<td>Name</td>
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<td>Concentration (%)</td>
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<td>α-Curcumene</td>
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Therefore, we examined the possibilities of the active compounds from turmeric that might inhibit Akt1 protein by using \textit{in silico} analysis.

**Four compounds inhibit Akt1 activity by molecular docking**

Akt1 is a protein that is overexpressed in breast cancer cells. This protein has a vital role in proliferation, metabolism, anti-apoptosis, and cell survival. Akt is divided into three subtypes, namely Akt1, Akt2, and Akt3, each of them has different roles. The Akt1 plays a role in the proliferation of T47D cells. Therefore, Akt1 becomes the focus target. Akt1 phosphorylates its substrate by binding to ATP in the kinase domain and then transferring the phosphate to the substrate. Therefore, one strategy to inhibit the activity of Akt1 is by blocking the bond between Akt1 and ATP. AZD5363 is a drug that can inhibit the activity of Akt1 by blocking the bond between Akt1 and ATP; hence, it is called a competitive ATP inhibitor compound. Therefore, AZD5363 was used as a positive control in this docking method.

Based on the binding site, the docking results showed four compounds bound to the ATP binding pocket area: curcumin, α-curcumene, Ar-turmerone, and caryophyllene oxide. Based on binding energy, curcumin and caryophyllene oxide had lower binding energy than AZD5363. Therefore, these two compounds had a high potential to inhibit the activity of Akt1 (Table 2). Moreover, the binding position of the ligands into Akt1 was similar to the AZD5363 (positive control) (Figure 2).

**The stability of complexes Akt1-ligands**

According to molecular dynamics indicated, the RMSD of the Akt1-ligands complexes was below 3 Å, which means that all complexes were still in a stable range (Figure 3A). The most stable ligand conformation was caryophyllene oxide followed by Ar-turmerone, AZD5363, curcumin, and α-curcumene (Figures 3B and 3C), as it is known that stable protein in the simulation could be determined from the RMSD value below 3 Å. However, the AZD5363 had the most stable

<table>
<thead>
<tr>
<th>Table 2. The binding affinity of Akt1 with the ligands.</th>
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<td><strong>Compounds</strong> &amp; <strong>Binding affinity (kcal/mol)</strong></td>
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<tr>
<td>AZD5363 (Inhibitor) &amp; -6.76</td>
</tr>
<tr>
<td>Curcumin &amp; -7.13</td>
</tr>
<tr>
<td>α-Curcumene &amp; -6.15</td>
</tr>
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<td>Ar-turmerone &amp; -6.1</td>
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<tr>
<td>Caryophyllene oxide &amp; -7.72</td>
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</tbody>
</table>

**Figure 2. Ligand binding position on Akt1.** The curcumin, α-curcumene, Ar-turmerone, and caryophyllene oxide have the same binding position as that of Akt1 with AZD5363.
movement in the receptor compared with the other ligands, followed by curcumin (Figure 3D). The RMSF value represents a shift in each amino acid residue that affects protein flexibility. High RMSF values indicate high flexibility during the simulation and stable residues during simulation. The simulation results showed that the residues that play an essential role in the ligand-binding site, such as Trp80, Tyr264, Lys268, and Lys272, were stable during the simulation (Figure 3B).

Discussion

We have examined the active compound of the ethanolic turmeric extract by using LC-HRMS and showed the extract containing curcumin, ferulic acid, 2-hydroxycinnamic acid, (E,E)-\(\alpha\)-farnesene, \(\alpha\)-curcumene, Ar-turmerone, caryophyllene oxide, and \(\beta\)-cymene. From the phenolic group, ferulic acid could inhibit the proliferation of HeLa and CaSki cells (cervical cancer cell line), and 2-hydroxycinnamic acid had anticancer activity in various types of human pancreatic cancer cells line. Meanwhile, in the terpenoid group, such as Ar-turmerone, \(\alpha\)-curcumene, and caryophyllene oxide, \(\alpha\)-curcumene was reported to induce apoptosis in SiHa cell line through mitochondrial cytochrome c. Ar-turmerone also induced apoptosis of some leukemia cell lines. Caryophyllene oxide could inhibit the proliferation of prostate and breast cancer cell lines. Among these compounds, curcumin is the most widely reported to have the best anticancer activity. Curcumin, Ar-turmerone, and caryophyllene oxide were also reported to be able to suppress the signaling of Akt, yet there is no explanation about the interaction between these compounds and PKB 1 protein (Akt1). However, future studies need to be conducted to evaluate the effect of \(C.\ longa\) crude extract on Akt1 activity.

The results of molecular docking and dynamics showed that compounds from turmeric have the potential to inhibit Akt1 activity through competitive inhibitory ATP mechanisms. Akt1 could inhibit caspases 3, 8, and 9 activities that play a role in apoptosis pathways. This protein is central in the PI3K/Akt pathway that shows overactivity in ER\(^+\) or HER\(^+\) type breast cancer cells. Previous studies mentioned that Akt1 was overexpressed in T47D cells. Overexpressed Akt1 will prevent cells from undergoing apoptosis. Therefore, apoptosis can be induced by inhibiting the activity of Akt1. Akt1 phosphorylates its substrates by binding to ATP in the kinase domain and then transferring the phosphate to the substrate. Therefore, one strategy to inhibit the activity of Akt1 is by blocking the bond between Akt1 and ATP. Previous studies showed that inhibition of Akt1 by competitive ATP inhibitors can inhibit the proliferation of various hepatocellular carcinoma cell lines. Akt1, which is inhibited by the compound through an ATP competitive inhibitor mechanism, suppresses the proliferation of malignant pleural mesothelioma cell lines. In previous studies, curcumin decreased Akt expression and inhibited invasion, metastasis, and induced apoptotic cell death of T47D breast cancer cells. In addition, Ar-turmerone attenuates breast cancer cell invasion by blocking the PI3K/Akt signaling pathway. Previous studies suggested that inhibition of Akt1 activity decelerates growth progression, migration, invasion, colony formation and induces apoptosis of breast and lung cancer cell lines. Downregulating Akt1 also inhibits the proliferation of...
Colorectal cancer cells and non-small-cell lung carcinoma (NSCLC). Compounds in turmeric might interact with Akt2 and Akt3, but we focus on Akt1 because the protein has an important role in anti-apoptosis and cell cycle regulation. A decrease in Akt1 expression will disrupt the cMyc-Akt1-p38 MAPK signaling pathway resulting in apoptosis of cancer cells. Meanwhile, Akt2 is more likely to induce tumor migration and invasion via the Akt2-PKM2-STAT3/NF-kB axis. Previous studies mentioned that Akt2 silencing in A549 and H1299 cell lines did not affect their apoptosis. Meanwhile, not much is known about the role of Akt3 in breast cancer. AZD5363 is a positive control in this molecular docking to assess the binding affinity and binding site of turmeric compounds interacting with Akt1. As a result, curcumin and caryophyllene oxide has the potential to act as Akt1 inhibitors.

Further investigation on breast cancer cell line T47D suggested that the extract strongly inhibits the cancer cell growth by inducing apoptosis and cell cycle arrest in the G1 phase. Akt1 has an important role in the cell cycle and this study showed that the cells arrested in the G0/G1 because of extract treatment, we speculated that the extract acted on Akt1 inhibition. The docking analysis showed four compounds, i.e., curcumin, α-curcumene, Ar-turmerone, and caryophyllene oxide, bound to the ATP binding pocket of Akt1. Furthermore, the molecular dynamic analysis suggested that the binding of the active compounds to Akt1 was very stable. The ligand-binding site such as Trp80, Tyr264, Lys268, and Lys272 was stable during the simulation. The data indicated that the ethanolic turmeric extract has an anticancer activity by inhibiting the Akt1 pathway. This prediction corresponded with a previous report that the inhibition of Akt1 could inhibit the PI3K/Akt pathway, which resulted in cell arrest in G1. Curcumin could inhibit the MCF-7 breast cancer cell line cycle in the G1 phase. Turmeric extract inhibited proliferation and induced apoptosis of breast cancer cuboid epithelial cells. However, the combination of a single compound between curcumin and other components on toxicity, apoptosis, and cell cycle in T47D cells was not carried out. Thus, further research can test the anticancer activity of the compounds contained in turmeric.

However, this study requires further studies to support the data that has been obtained. Cytotoxicity assay needs to be continued on normal cells to determine the selectivity of the extract. All assays need to be continued in a time-dependent manner such as for 48 and 72 hours and use key identified curcuminoid. The molecular docking and dynamic need to be validated and confirmed using western blot.

**Conclusions**

Turmeric ethanol extract has an anticancer activity on the T47D cancer cell line with an IC₅₀ value of 26.36 ± 1.55 µg/mL. The extract induced apoptosis and cell cycle arrest in the G1 phase. The docking analysis showed that the active compounds of the extract, such as curcumin, Ar-turmerone, caryophyllene oxide, and α-curcumene, were able to bind into the ATP-binding pocket of Akt1 that might inhibit the protein activity and induce cell cycle arrest.

**Data availability**

**Underlying data**


This project contains the following underlying data:

- Data file: *Curcuma longa* data.xlsx (Raw data of DPPH, apoptosis, cells cycle, and cytotoxicity)
- Data file: docking result of pkb vs ar turmerone_.pdb
- Data file: docking result of pkb vs azd1.pdb
- Data file: docking result of pkb vs crn1.pdb
- Data file: docking result of pkb-Caryophyllene oxide_.pdb
- Data file: molecular dynamic of Akt1 vs a-curcumene_analysis.tab
- Data file: molecular dynamic of Akt1 vs Curcumin_analysis.tab

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).
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