



Anticancer effects of *Curcuma zedoaria* (Berg.) Roscoe ethanol extract on a human breast cancer cell line

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Abstract

Breast cancer is a malignant cancer with annually increasing incidence and mortality rates. The anticancer effect of *Curcuma zedoaria* (Berg.) Roscoe ethanol extract (ECz) on T47D breast cancer cells was investigated in vitro. The ECz contains phenolic and flavonoid compounds that may play a critical role in anticancer activity. The viability of the treated T47D cells was significantly reduced compared to that of the control after treatment with ECz. The extract was able to induce apoptosis, upregulate B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax), caspase-9, and caspase-3 protein expression levels, followed by a decrease in Bcl-2 levels and loss of mitochondrial membrane potential. In addition, the ECz also inhibited colony formation and cell migration. Signaling pathway analysis confirmed that ECz decreased the phosphorylated p38 mitogen-activated protein kinase levels. Taken together, the data suggest that ECz might be beneficial as a therapeutic agent for breast cancer.

Keywords Breast cancer · Cell colony · Cell migration · *C. zedoaria* · Protein expression

Introduction

Breast cancer is the most common cancer diagnosed in women and the fifth leading cause of death in women after lung cancer, colon cancer, liver cancer, and stomach cancer. Lifestyle and socio-cultural changes due to economic growth and the high rate of delayed pregnancy increase the risk factors for breast cancer in developed countries (Hashim 2015; Sung et al. 2021). The World Health Organization data show that in 2020, the incidence of breast cancer is the highest (11.7%), with a mortality rate of 6.9%. Since the last 5 years, Asia has been ranked first for the incidence and mortality rates due to breast cancer (Barbieri 2019; DeSantis et al. 2015; Shah et al. 2014). Financial constraints, untrained health personnel, lack of resources, and inadequate infrastructure are factors that cause ineffective

breast cancer treatment in various low- and middle-income countries. This is in contrast to studies that have shown that the optimal treatment of breast cancer depends on proper diagnosis followed by organized treatment (Ginsburg et al. 2020; Mutebi et al. 2020). Alternative medicine using various herbs is the choice of many breast cancer patients because it can be obtained at low prices. However, the use of herbal medicine for cancer therapy still needs to be proven by scientific evidence.

Zingiberaceae has long been known as an herb used in traditional medicine (Sharifi-Rad et al. 2017). This group of plants is known to have anticancer activity because it contains various compounds, including terpenoids, flavonoids, sesquiterpenes, and phenylpropanoids (Carson and Hammer 2011). *Curcuma zedoaria* (Berg.) Roscoe is a member of Zingiberaceae, which is known to have anticancer properties and has been studied for a long time (Gao et al. 2014; Lobo et al. 2010; Seo et al. 2005; Shin and Lee 2013). In silico research indicated that compounds from *C. zedoaria* might inhibit C-X-C chemokine receptor type 4 (CXCR4), which plays an important role in cancer development (Fitriana et al. 2020). Another study showed that *C. zedoaria* extract could inhibit the growth of esophageal cancer (Hadisaputri et al. 2015).

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The active compounds from *C. zedoaria*, such as curcumin, curcuzedoalide, and isocurcumenol, are able to induce cytotoxic activity in various types of cancer cells (Jung et al. 2018; Lakshmi et al. 2011; Shin and Lee 2013). Various studies have been conducted to determine the overall mechanism of anticancer activity (Gao et al. 2014; Hadisaputri et al. 2015; Rahman et al. 2013a). However, there are still no reports regarding the possible selective toxicity of the extract in breast cancer cells. Therefore, this study aimed to provide additional information on the anticancer activity of *C. zedoaria*, particularly T47D breast cancer cells, and elucidate the molecular mechanism.

Experimental

Plant material, extraction, and preparation of drug

C. zedoaria (Berg.) Roscoe was collected from Balai Materia Medica, Batu, East Java, Indonesia, with a registration number. The extract was obtained by the maceration method, and 96% ethanol was added to the rhizome powder (1:2, w/v). The maceration process was carried out for 3 d, with solvent replacement at room temperature. The filtrate was then concentrated by a rotary evaporator (Buchi R-114) at 55 °C. The stock solution of the crude extract was prepared by dissolving 128 mg/mL in dimethyl sulfoxide. The stock solution was used for further analysis.

Cell line and cell culture

T47D breast cancer cell line was cultured in Roswell Park Memorial Institute medium (RPMI, Gibco). TIG-1 was used as a control to determine the toxicity level of ECz in normal cells and was cultured in minimum essential medium (MEM, Gibco). The growth medium was supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin–streptomycin (Gibco). The cells were incubated at 37 °C in a 5% CO₂ incubator.

Toxicity assay

WST-1 assay was used to determine the toxicity level of the T47D breast cancer cell line treated with ECz. A total of 7.5×10^3 cells were seeded in 96-well plates and incubated for 24 h. The cells were treated with 20, 40, and 80 µg/mL of ECz and incubated at 37 °C in a 5% CO₂ incubator. The treatment medium was replaced with a medium containing 5% WST-1 (Rosche, Sigma-Aldrich, USA) and incubated for 30 min. Absorbance was measured at 450 nm using Elx808™ (BioTek Instrument, USA).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay

The DPPH method was used to evaluate ECz's antioxidant activity. Standard and extract with different concentrations (31.25, 62.5, 125, and 250 ppm) were added to 100 µL of DPPH in ethanol solution (0.4 mM) in 96-well plates and incubated for 30 min at room temperature. Absorbance was measured at 490 nm using an enzyme-linked immunosorbent assay (ELISA) reader.

Total flavonoid and total phenolics determination

Aluminum chloride colorimetric assay and the Folin–Ciocalteu assay were used to measure the total flavonoid and phenolic contents from the ECz, respectively (Chatatikun 2013; Jing et al. 2015). For the total flavonoid content, 50 µL of the sample was added to 10 µL of AlCl₃ (10%, w/v), followed by 150 µL of 96% ethanol solution. Then, 10 µL of CH₃COONa (1 M) was added to the mixture in 96-well plates and incubated for 40 min at room temperature and dark conditions. The absorbance was measured at 405 nm using an ELISA reader.

For the total phenolic content, 100 µL of the sample was added to 1.0 mL of Folin–Ciocalteu reagent, and the mixture was incubated for 5 min. Then, 1.0 mL of Na₂CO₃ (7.5%, w/v) was added and the mixture was incubated for 90 min at room temperature and dark conditions. The total phenolic content was measured at 725 nm by spectrophotometry.

Cell apoptosis assay

Annexin V-FITC/PI, SYTO 9/PI, and rhodamine 123 were used for apoptotic cell detection. A total of 7.5×10^4 cells were seeded in 24-well plates and incubated for 24 h at 37 °C in a 5% CO₂ incubator. The cells were treated with ECz at the concentration 102 µg/mL and incubated under the same condition. Cells were trypsinized, harvested, resuspended, and washed with PBS. The cells were centrifuged, the supernatant was discarded, and the cells resuspended with 50 µL Annexin V/PI (1:2; Invitrogen, USA) and incubated for 30 min at dark and cold conditions. Then, 400 µL of PBS was added, and the mixture analyzed by flow cytometry (BD Biosciences, USA). Data were analyzed by CellQuest software (BD Biosciences).

A total of 7.5×10^4 cells were seeded on coverslip in the 24-well plates. After 24 h, cells were treated with ECz at the concentration 102 µg/mL and incubated at 37 °C in a 5% CO₂ incubator. The cells were washed with PBS and

incubated with 200 μL SYTO 9/PI in PBS (1:2500, Invitrogen L7012) for 30 min at room temperature. Thereafter, the cells were washed with PBS and visualized by confocal laser scanning microscopy.

For the mitochondrial apoptotic pathway, rhodamine 123 (2 μM) was added to each well and incubated for 1 h at 37 °C in a 5% CO_2 incubator. The cells were trypsinized, harvested, and centrifuged for 5 min at 10 °C and 2,500 rpm. The cells were washed with PBS, resuspended with basal medium, incubated for 30 min at room temperature, and analyzed by flow cytometry (BD Biosciences). Data were analyzed by CellQuest software (BD Biosciences).

Morphological changes using phase-contrast inverted microscopy

Morphological changes in the apoptotic cells were performed by phase-contrast inverted microscopy. The cells were seeded and treated with different concentrations of ECz (51, 102, and 204 $\mu\text{g}/\text{mL}$) and incubated at 37 °C in a 5% CO_2 incubator. Then, the treatment medium was discarded, and the cells were washed with PBS and observed by phase-contrast inverted microscopy at 20 \times magnification.

Wound healing assay

A total of 3×10^5 cells were seeded in 24-well plates and incubated for 24 h. The monolayer cells were scratched by the sterile yellow tip, and detached cells were removed by washing PBS. PBS was removed and replaced with the treatment medium. The scratch closure was observed three times (0, 16, and 24 h) by phase-contrast inverted microscopy at 10 \times magnification.

Colony-forming assay

The colony-forming assay was performed based on a previous study with modification. Cells were seeded in 24-well plates with a density of 7.5×10^4 cell/well. After 24 h, the cells were treated with different concentrations of ECz (51, 102, and 204 $\mu\text{g}/\text{mL}$) and incubated for 24 h. The cells were harvested and reseeded in six-well plates, with densities of 125, 250, 500, 750, 1000, and 1500 cells/well and incubated for 8 days. Finally, the colonies formed were fixed using Methanol/Acetic acid (1:1, v/v) solution and stained with 0.5% crystal violet. Colonies consisting of more than 50 cells were counted.

Apoptosis-related protein assay

A total of 7.5×10^4 cells were seeded and treated with ECz at the concentration 102 $\mu\text{g}/\text{mL}$. The cells were trypsinized, harvested, and centrifuged. Then, the cells were incubated

with 50 μL of fixation buffer for 30 min on ice. The cells were centrifuged and resuspended with 50 μL of anti-Bax antibody (sc-20067), anti-caspase-3 antibody (sc-7272), and anti-caspase-9 antibody (sc-17784) and incubated for 30 min in dark and cold conditions. Four hundred microliters of PBS was added, and the mixture was analyzed by flow cytometry (BD Biosciences). Data were analyzed by CellQuest software (BD Biosciences).

Western blot analysis

A total of 2.2×10^6 cells were seeded in 100-mm dishes and treated with the IC_{50} (102 $\mu\text{g}/\text{mL}$) of ECz. The cells were harvested, centrifuged, resuspended with RIPA buffer, and incubated on ice for 20 min. Then, the cells were centrifuged at 12,500 rpm for 15 min at 4 °C. Protein samples were loaded into 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred into a polyvinylidene difluoride membrane. The membrane was incubated in 5% skim milk blocking solution for 1 h at room temperature. After that, the membrane was washed three times with TBST and incubated with the primary antibody (MAPK sc-7972 and Bcl-2 sc-7382) (1:1000) overnight at 4 °C. The membrane was washed with TBST thrice, incubated with HRP Goat anti-mouse IgG secondary antibody (Rockland) (1:2000) for 1 h at room temperature, and washed with TBST. The protein bands were detected by ImageQuant LAS 500.

Active compounds analysis

Active compounds contained in ECz were analyzed by liquid chromatography-high-resolution mass spectrometry. ECz was diluted by adding 0.1% ethanol solvent with a final volume of 1500 μL . The sample was mixed, vortexed, and centrifuged for 2 min at 6000 rpm. The sample was filtered by a 0.22 μm syringe filter. The sample was processed into an autosampler (Thermo Fisher Scientific Inc., USA) and injected in liquid chromatography-high-resolution mass spectrometry (LC-HRMS, Thermo Scientific Dionex Ultimate™ 3000 RSLCnano with microflow meter; Thermo Fisher Scientific Inc.).

The analysis of LC-HRMS, the column oven maintained at 30 °C, 100 mL of volume was injected, the analytical column was Hypersil GOLD aQ (Hypersil GOLD™ aQ C18 Polar Endcapped HPLC Columns; Thermo Fisher Scientific Inc.) 50 \times 1 mm \times 1.9 μm particle size and solvent A = 0.1% formic acid in water and solvent B = 0.1% formic acid in acetonitrile were used. The analytical flow rate was 40 mL/min with a run time of 30 min. The positive ion mode detection was performed using a mass spectrometer (Q Exactive™ Orbitrap Mass Spectrometers; Thermo Fisher Scientific Inc.).

Data analysis

Data were represented as the mean \pm standard deviation (SD) of the three independent experiments. Statistical comparisons of the control (untreated cells) to the cells were treated with different concentrations of ECz. The analysis of the Western blot and the scratch images were performed using ImageJ. Statistical analysis of the data using independent sample *T*-test and one-way analysis of variance was considered significant at $p < 0.05$ and $p < 0.01$.

Results

Cytotoxic effect, phytochemical screening, and antioxidant activity of ECz

ECz cytotoxicity activity was observed by comparing the number of live cells between the control and treatment groups after incubation for 24 h. ECz was significantly selective in inhibiting the growth of the T47D cell line than with the normal cell line TIG. At the highest concentration, it was observed that the percentage of live T47D cells was significantly lower than that the TIG cells (Fig. 1a). The IC₅₀ of ECz in the T47D cells was three times lesser than that in the TIG cells, with values of 101.9 ± 0.9 $\mu\text{g/mL}$ and 341 ± 2.6 $\mu\text{g/mL}$, respectively. The anticancer activity of ECz may be due to its phenolic and flavonoid contents; there are 10.11 ± 0.4 mgGAE/g and 0.77 ± 0.03 mgQE/g, respectively. ECz also has antioxidant activity, with an IC₅₀ value of 590.630 ± 19.8 ppm.

ECz induces apoptosis in human breast cancer cell line

Programmed cell death, known as apoptosis, is a normal physiological process to eliminate unwanted cells. Apoptotic events in the early stages are characterized by translocation of the phosphatidylserine (PS) membrane from the inner side of the plasma membrane to the outer side of the plasma membrane. Cells undergoing apoptosis can be detected by annexin V-FITC and PI using flow cytometry. This analysis can distinguish cells into four groups, namely, viable cells, early apoptosis, late apoptosis, and necrotic cells. Early apoptosis is characterized by translocation of PS to the outside of the plasma membrane so that annexin V that has been conjugated with FITC bind to PS and shows green fluorescence. PS translocation is followed by the loss of plasma membrane integrity so that PI enters the cell. PI will bind to DNA, indicating that the cell has undergone late apoptosis (Rieger et al. 2011; Looi et al. 2013; Assays et al. 2016). This study showed that the administration of ECz treatment at a concentration of 102 $\mu\text{g/mL}$ caused cells to

undergo early apoptosis and late apoptosis, 67.6% and 6.1%, respectively, compared to the control (Fig. 1c, d).

SYTO9 and PI counterstain is often used to identify apoptotic events in cells. SYTO9 can enter living cells bind to DNA and RNA so that it emits green fluorescent. Meanwhile, PI can only enter cells that have lost their membrane integrity, bind to DNA and RNA so that they emit red fluorescence. This indicates that cells undergoing apoptosis will emit red fluorescence (Stiefel et al. 2015; Rosenberg et al. 2019). Administration of ECz (102 $\mu\text{g/mL}$) caused cells to undergo apoptosis, as evidenced by the confocal laser scanning microscopy observations that red fluorescent cells were present. This result was very different from that of the control, in which none of the cells in emitted red fluorescence (Fig. 1e).

Disruption of mitochondrial membrane potential (MMP) is one of the characteristics of cells undergoing apoptosis. MMP functions to regulate the permeability and selectivity of the mitochondrial membrane to various substances to maintain the structure and function of the mitochondria. Rho-123 is a kit used to detect MMP interference (Zhang et al. 2016; Wang et al. 2021). Cells treated with ECz suffered mitochondrial damage after incubation for 24 h. The results showed that there was a significant decrease in fluorescence in the treated cells compared to that of the control (untreated cells). MMP disturbances increased with an increasing incubation time of the treatment. The flow cytometry results showed that the MMP disturbance in the cells incubated for 48 h was greater than that in the cells incubated for 24 h, 80.8% and 61.5%, respectively (Fig. 1f).

Moreover, the morphological changes of cells undergoing apoptosis were characterized specifically, including loss of asymmetry and plasma membrane attachment, plasma membrane blebbing, and condensation of the cytoplasm and nucleus. Observations with phase-contrast inverted microscopy showed an increase in the concentration of ECz, given the changes in the morphology of cells undergoing apoptosis. In addition, cells undergoing apoptosis lose their ability to adhere to cell culture plates (Fig. 2).

ECz activity on activation and inhibition of apoptotic-related proteins

Changes in protein expression levels associated with apoptosis are parameters that can be observed to explain the mechanism of apoptosis that occurs after cancer cells are treated with ECz. Changes in protein expression levels were determined by flow cytometry and western blotting analysis. As shown in Fig. 3a, treatment with ECz at a concentration of 102 $\mu\text{g/mL}$ for 24 h could increase the expression level of Bax, caspase-9, and caspase-3 proteins compared to untreated cells. Bax (80.1%), caspase-9 (55.1%), and caspase-3 (65.2%) experienced a very significant increase

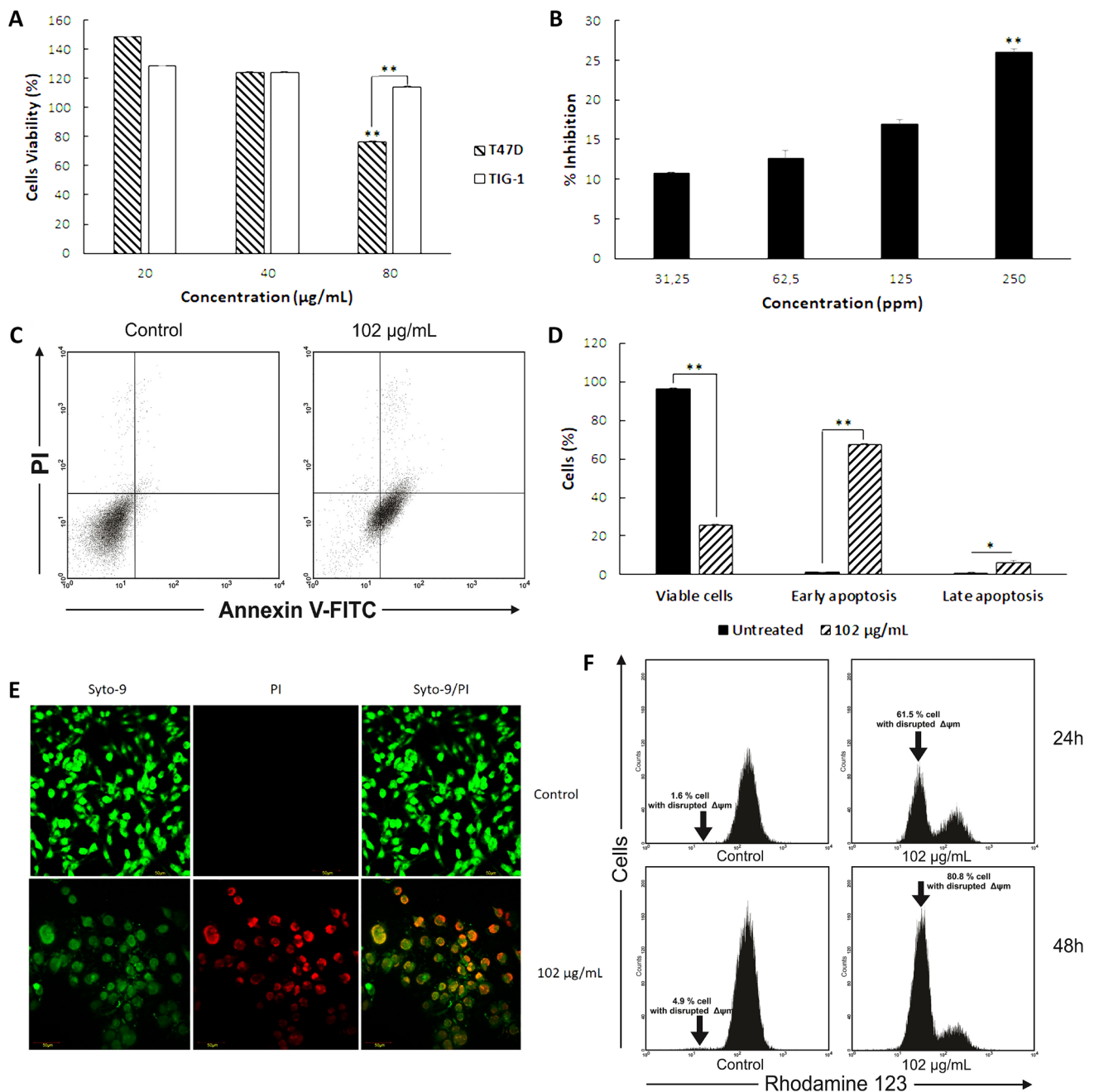


Fig. 1 Antioxidant and anticancer activities induced by ECz treatment. **a** Percentage of growth inhibition on T47D and TIG cells line. **b** DPPH scavenging activity of ECz. **c**, **d** Annexin V-FITC/PI assay by flow cytometry and histogram of viable cells and cells undergo-

ing apoptosis after ECz treatment. **e** Visualization of cells undergoing apoptosis by SYTO 9-PI staining. **f** Mitochondrial membrane disruption by Rho-123 staining

compared to that of the controls at 1.6%, 14.8%, and 16.6%, respectively (Fig. 3b).

p38 MAPK and Bcl-2 are also promising targets for cancer treatment. Western blotting analysis showed decreased

expression of p38 MAPK and Bcl-2 in the ECz-treated breast cancer cells. p38 MAPK protein was significantly decreased after 48 h of incubation in the treatment medium

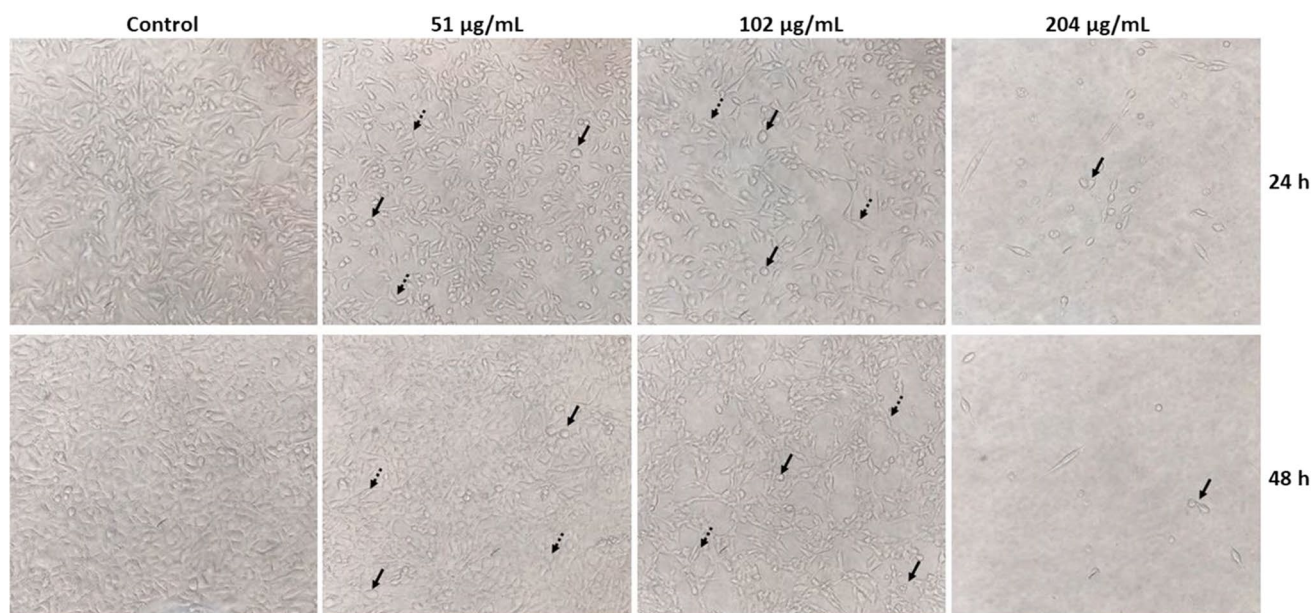


Fig. 2 Morphological changes of T47D breast cancer cells visualized by phase-contrast inverted microscopy. Arrow: T47D cells under normal conditions; dot arrow: T47D cells undergo dead

(Fig. 3c). In contrast to p38 MAPK, Bcl-2 levels significantly reduced at 24 and 48 h after treatment (Fig. 3d).

ECz inhibition of cell migration in different concentration

Breast cancer cell line T47D was treated with ECz at three different concentrations to investigate cell migration by the wound healing method. The percentage of migration area in the T47D cells after scratching was significantly decreased compared to that in controls after being treated for 16 and 24 h. Untreated or control cells significantly migrated, and after 24 h, it was seen that the migration area was more than 80%. The breast cancer cell line T47D treated with ECz at a concentration of 204 µg/mL significantly had the smallest migration area compared to those in the control and other treatments (Fig. 4).

Colonies formed in the presence of ECz

Colony-forming assay, known as clonogenic, is a test that aims to determine the viability of cells based on the ability of single cells to grow into colonies. The formed cell colony consists of at least 50 cells or more. This test is commonly used to determine the ability of cells to divide after treatment with cytotoxic agents and is used to determine the long-term effects of the treatment (Shin and Lee 2013; Mahdizade et al. 2019).

In this study, plating efficiency or colony-forming efficiency was examined after being treated with ECz with

different concentrations for 24 h. The results in Fig. 5 and Table 1 show that ECz treatment with a concentration of 204 µg/mL for 24 h significantly reduced the number of colonies formed compared to the number of colonies in the control. In addition, it was found that increasing the concentration of the treatment decreased the colony-forming ability of T47D cells (Fig. 6).

Active compounds analysis

The active compounds contained in ECz were analyzed by LC-HRMS. The results of the analysis showed that there were two main groups of compounds detected in the extract, namely curcuminoids and sesquiterpenoids. Bisdemethoxycurcumin and demethoxycurcumin are two active compounds of the curcuminoid group that play an important role as anticancer agents (Hsia et al. 2022; Luo et al. 2021). Five active compounds from the sesquiterpenoids group were identified in this study, namely curzerenone, bisacurone, zedoarondiol, zedoalactone C, and zedoarolide B (Fig. 7).

Discussion

Currently, many studies have been conducted to identify natural compounds that have potential as alternative cancer treatments. Besides that, it is hoped that the natural compounds developed have few side effects. *C. zedoaria* (Berg.) Roscoe has been reported to have anticancer activity (Hudaya et al. 2016; Rahman et al. 2013a). In this study,

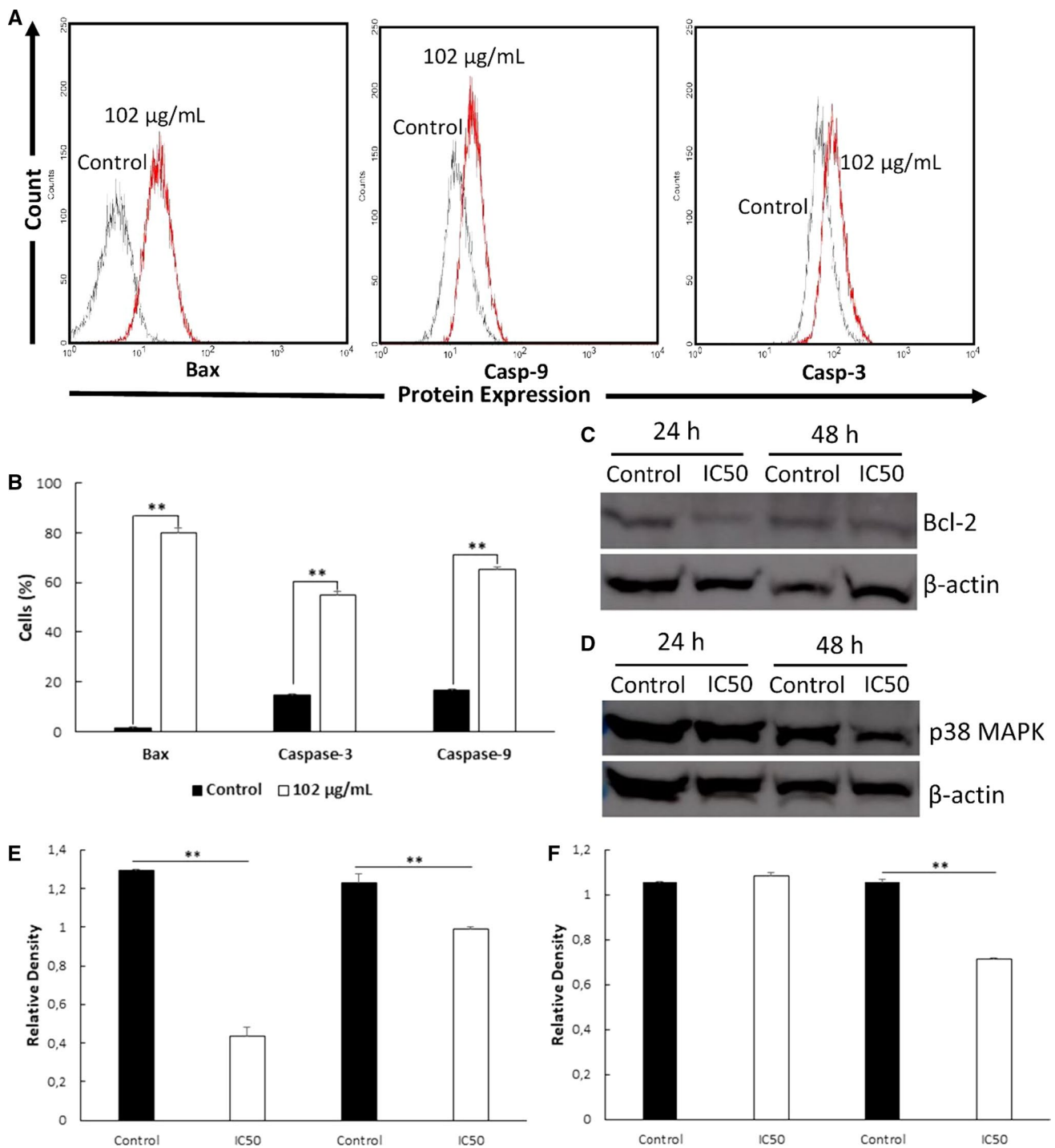


Fig. 3 Changes in the protein expression levels, an important role in inhibiting cell growth. **a** The expression of Bax, caspase-9, and caspase-3 was analyzed by flow cytometry. **b** Percentage of Bax, caspase-9, and caspase-3 expression. **c, d** The expression of p38 MAPK

and Bcl-2 was analyzed by an immunoblot assay. The value presented is representative of three independent experiments (mean \pm SD, $**p < 0.01$, compared to the control)

the anticancer effect of *C. zedoaria* (Berg.) Roscoe showed selective killing on the breast cancer cell line T47D—inducing apoptosis and inhibiting cell migration and colony formation. Cell viability was observed to determine the effect

of *C. zedoaria* in inhibiting the growth of T47D cells. *C. zedoaria* was significantly toxic to T47D cells compared to the normal cells TIG-1. The result also comparable with previous report that *C. zedoaria* showed inhibited the growth

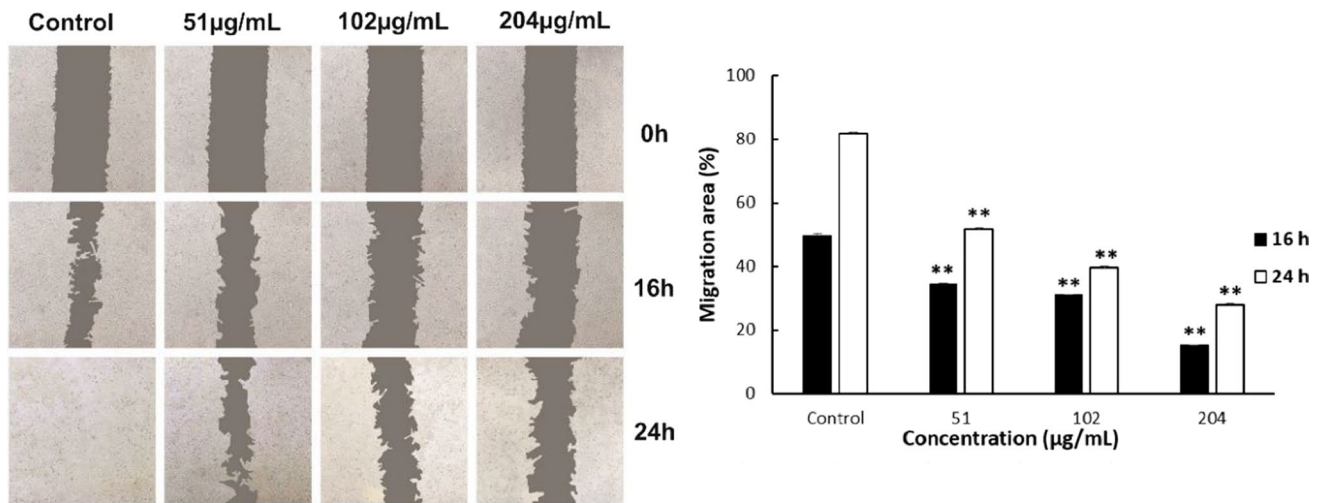


Fig. 4 Cell migration after treatment with different concentrations of ECz. Visualization of cell migration after 16 and 24 h of treatment and histogram of the migration area distance after scratching in three

independent experiments, wherein each treatment concentration was compared with the control at a significant level of $**p < 0.01$

Fig. 5 Images were taken 8 d after the initiation of colony-forming assay. Colonies generated when different densities of the T47D breast cancer cell line were used

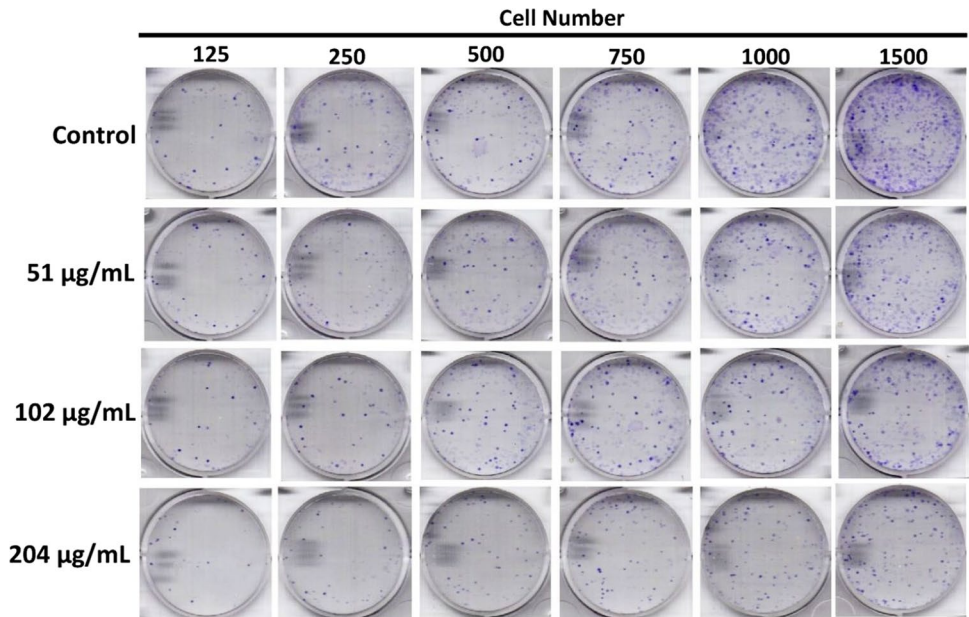


Table 1 The mean colony count of T47D cells after treatment with different concentrations of ECz

Treatment (µg/mL)	Colony count					
	125 Cells	250 Cells	500 Cells	750 Cells	1000 Cells	1500 Cells
Control	87.3	142	188	256	309	454
51	63.3	100.3	167.3	239.3	280	373.7
102	53.7	93.3	150.7	228	270	319.3
204	25.3	46.7	86.3	114.7	128.3	163

The colonies were counted after 8 days. The experiment was performed in triplicate

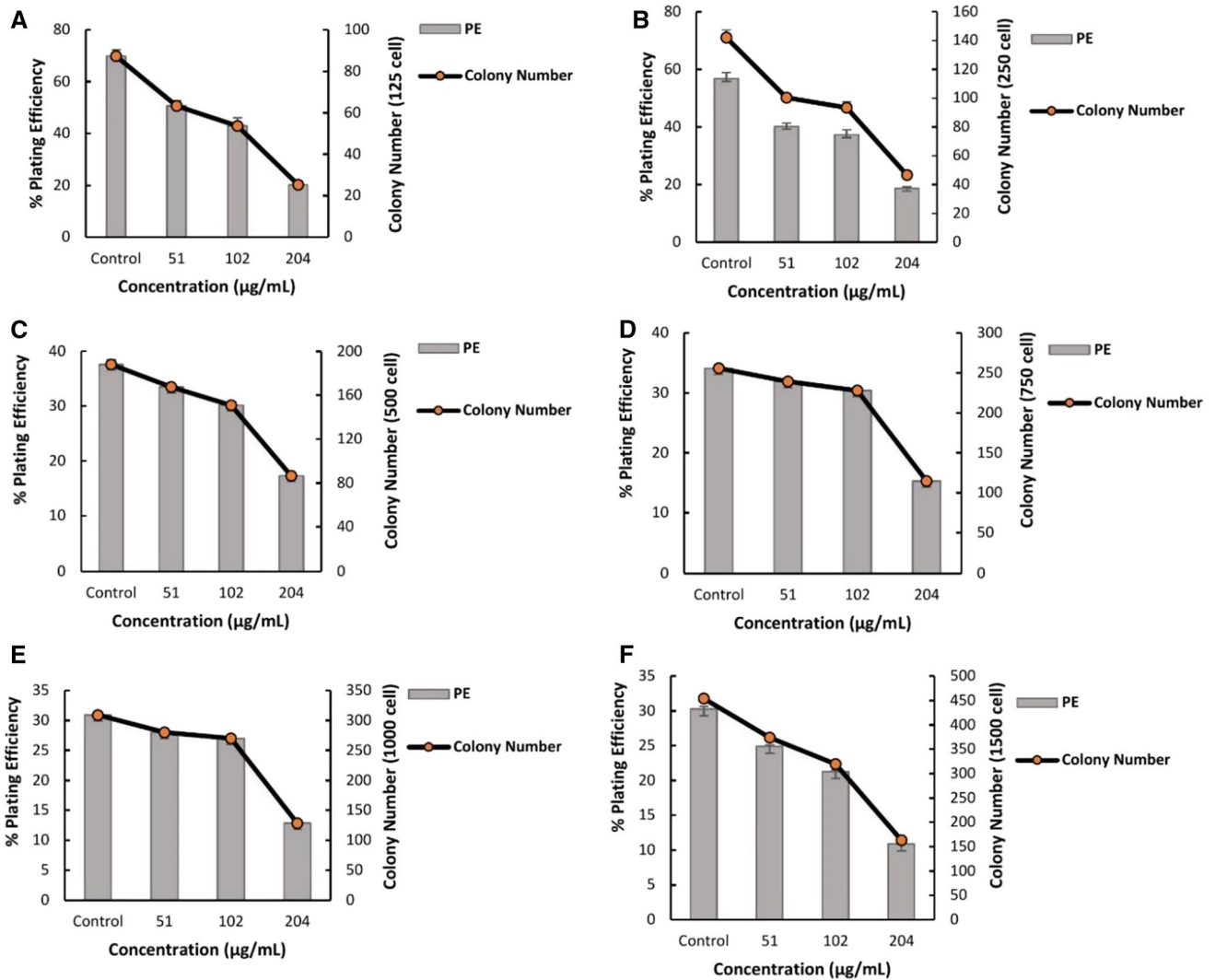


Fig. 6 The effect of ECz on different T47D cell numbers after 24 h of incubation. Values are expressed as mean \pm SD of three independent experiments

of H1299, A549 (Chen et al. 2013), MDA-MB-231 cell (Gao et al. 2014), and EAC cell line (Pal et al. 2015).

Administration of *C. zedoaria* at a concentration of 102 µg/mL significantly increased apoptosis processes on T47D cells. In this study, apoptosis characterized by cell membrane damage, loss of mitochondrial membrane potential, cell morphological changes, increased pro-apoptotic proteins and decreased anti-apoptotic protein. Apoptosis is a cell death mechanism that plays an important role in cancer development and is a target for many anticancer treatments (Kalid et al. 2010; Zhou et al. 2015). Apoptosis that occurs in this study is possible through extrinsic and intrinsic pathways. Mitochondria play an essential role in the process of intrinsic apoptosis controlled by the Bcl-2 family of proteins, consisting of pro-apoptotic and anti-apoptotic proteins such as Bax and Bcl-2 (Lei et al. 2012). Caspase

family proteins also play an important role in the process of apoptosis, including caspase-9 and caspase-3 (Halimah et al. 2015; Li et al. 2011; Zheng et al. 2016). Besides that, increased expression of p53 protein plays an important role in suppressing the development of cancer cells and is able to induce the process of apoptosis. In this study, expression of p53, Bax, caspase-9, and caspase-3 proteins were increased and decreased Bcl-2 protein expression in T47D cells. The data correspond to the previous report that *C. zedoaria* can induce apoptosis of MDA-MB231 cells through the modulation of Bax and Bcl-2 proteins (Lourembam et al. 2019) and also induce expression of caspase-9, caspase-3, and p53 (Wang et al. 2018). The p53 and caspase family proteins play an important role in the process of apoptosis (Li et al. 2011; Halimah et al. 2015; Zheng et al. 2016). This study showed that ECz treatment significantly decreased

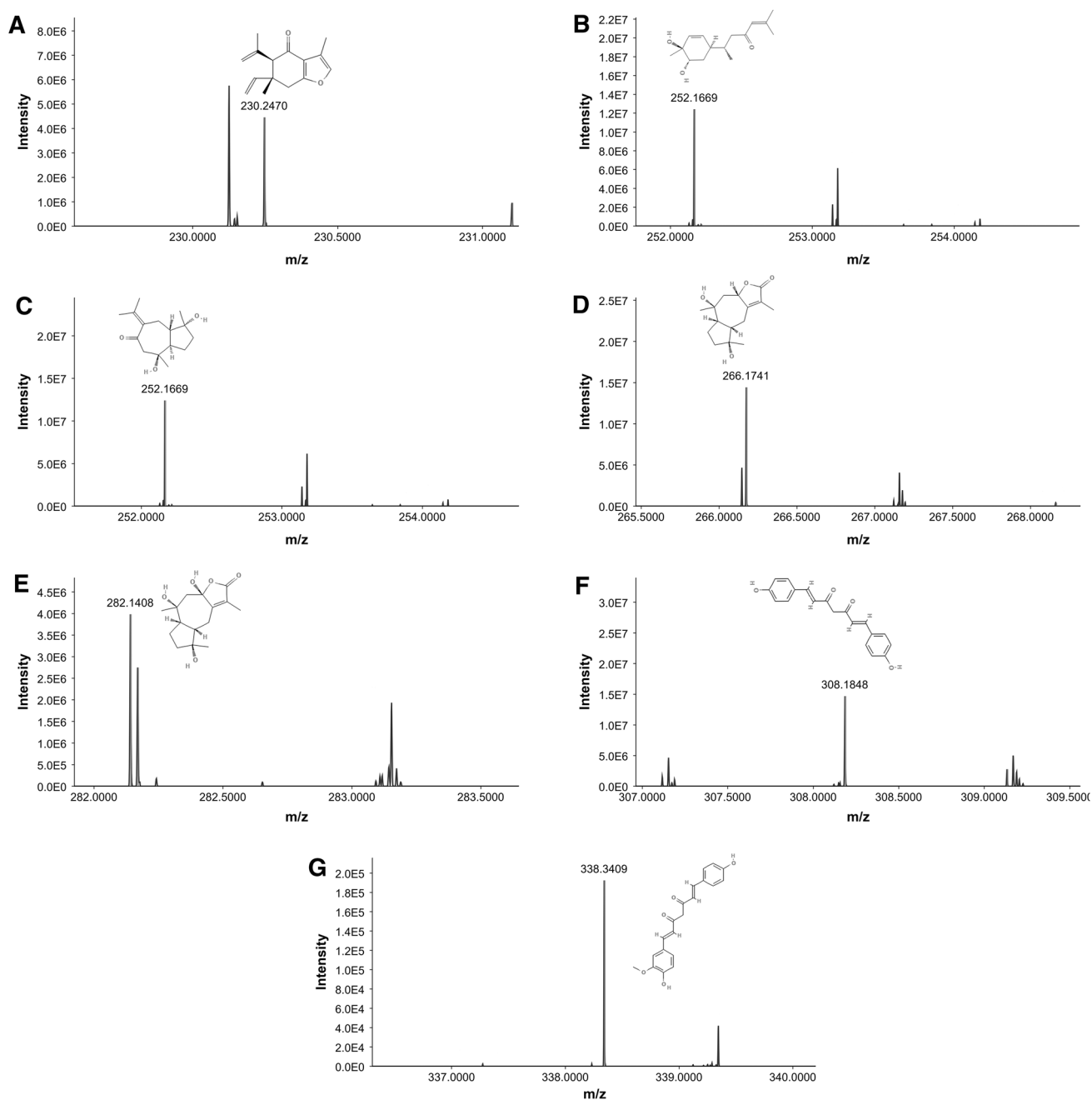


Fig. 7 LC-HRMS spectrum of active compounds in ECz. Seven mass spectra were obtained and shown **a–g**. **a** Curzerenone, **b** Bisacurone, **c** Zedoarondiol, **d** Zedoalactone C, **e** Zedoarolide B, **f** Bisdemethoxycurcumin, and **g** Demethoxycurcumin

p38 MAPK expression (Fig. 2d). Activated MAPK causes phosphorylation of p38, thereby activating transcription factors and promoting apoptosis (Ning et al. 2016). p38 MAPK is a vital signaling pathway that can activate Bax after its translocation to the mitochondria (Jiang et al. 2017).

The seven compounds identified in this study may have anticancer effects by inducing apoptosis. Bisdemethoxycurcumin, a curcuminoid group, can induce apoptosis by increasing the expression of cytochrome C and Bax and

decreasing the expression of Bcl-2 in NSCLC and GBM 8401/luc2 cells (Hsia et al. 2022; Xiang et al. 2020). Bisacurone and curzerenone, the identified sesquiterpenoids, are known to induce apoptosis by inhibiting AKT phosphorylation and Bcl-2 expression, increasing Bax expression and activating caspase-3 (Sun et al. 2008; Rahman et al. 2013a, b; Zheng et al. 2019). Zedoarondiol and Zedoarolide B are known to have toxic effects on human cancer cells, but there has been no further research on

the anticancer effects of these two compounds (Al-Amin et al. 2021).

Inhibition of migration and formation of new colonies of cancer cells is one of the parameters observed in the development of anticancer drugs. The high mortality rate in cancer patients is due to the presence of cancer cells that metastases and can develop in other parts of the body (Shah et al. 2018; Aglund et al. 2004; Chun and Kim 2013). Previous studies have shown that cell migration is regulated by a family of MMPs proteins capable of degrading the basement membrane and extracellular matrix (Liao et al. 2012; Li et al. 2016). Identification of colony formation and cell migration was used further to evaluate the anticancer effect of ECz on T47D cells. The results showed that ECz significantly inhibited colony formation and migration of T47D breast cancer cells. The results of previous studies showed that bisdemethoxycurcumin and demethoxycurcumin play an important role in inhibiting MMP-2 and MMP-9 which play an important role in cell migration (Xiang et al. 2020).

Conclusions

Research shows that ECz has a significant anticancer effect on breast cancer cells. The results indicated that ECz was able to inhibit apoptosis, colony formation, and cell migration by regulating the expression of Bax, caspase-9, and caspase-3 and p38 MAPK pathway. This research can also be used as a clinical basis for the development of cancer drugs using *C. zedoaria*.

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Declarations

Conflict of interest All Authors of manuscript entitle “Anticancer effects of *Curcuma zedoaria* (Berg.) Roscoe ethanol extract on a human breast cancer cell line” declare that he/she has no conflict of interest.

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