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RESEARCH ARTICLE

Tacca chantrieri Rhizome Extract Induces Apoptosis and Inhibits Colony Formation and Migration in Triple-Negative, HER2+, and ER+ Breast Cancer Cell Lines

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Abstract

Breast cancer is the most common cancer in females worldwide, and standard chemotherapy often causes significant side effects. *Tacca chantrieri*, a plant used in traditional medicine, contains bioactive compounds with anti-cancer properties. This study aimed to evaluate the anti-cancer activity of *T. chantrieri* rhizome extract (TCE) on breast cancer cell lines. The rhizomes of *T. chantrieri* were extracted with 95% ethanol and used to treat MDA-MB-231, MCF-7, and SK-BR-3 breast cancer cell lines. Cell viability was assessed using the MTT assay. Apoptosis was evaluated by annexin V and propidium iodide staining, while colony formation and cell migration were assessed using clonogenic and wound healing-scratch assays, respectively. The results showed that TCE significantly reduced cell viability, induced apoptosis, and inhibited colony formation and migration. In conclusion, TCE exhibits anti-cancer activity against breast cancer cells, highlighting its potential for further development as an alternative treatment for breast cancer.

Keywords: *Tacca chantrieri*, Breast cancer, Anti-cancer agent, Apoptosis, Cell migration inhibition

Introduction

Breast cancer is the most commonly diagnosed cancer among women worldwide, with 2,295,686 new cases and 665,684 deaths reported in 2022 [1]. Although chemotherapy remains a standard treatment, it is often associated with severe side effects, including toxicity, drug resistance, and a decline in the quality of life of the patient [2]. As a result, natural products have garnered increasing attention as alternative therapeutic options due to their potential benefits, such as reduced adverse effects and the ability to target multiple cancer-related pathways [3,4]. Therefore, the development of novel bioactive compounds derived from natural sources presents a promising strategy for safer and more effective breast cancer treatment.

Tacca chantrieri, known as the "black bat flower," is a herbaceous plant found in the tropical forests of several Southeast Asian countries [5,6]. The rhizome of this plant has been traditionally used in medicine to alleviate muscle pain, abdominal discomfort, food poisoning, skin diseases, burns, diarrhea, stomach ulcers, and inflammation [7-10]. Studies have revealed that *T. chantrieri* contains several bioactive compounds with anti-cancer activities, including saponins, taccalonolides, withanolides, diarylheptanoids, and diarylheptanoid glucosides [11-13]. These compounds have demonstrated cytotoxic effects against various cancer cell types, highlighting *T. chantrieri* as a promising candidate for cancer treatment. Notably, an ethanol extract of *T. chantrieri* rhizome has been found

to exhibit anti-cancer activity and enhance the effects of cisplatin in cholangiocarcinoma cells [14].

While the anti-cancer potential of *T. chantrieri* has been demonstrated in certain cancer types, its effects on breast cancer cells remain underexplored. This study aims to evaluate the anti-cancer activity of *T. chantrieri* extract (TCE) in breast cancer cell lines. The cytotoxic effect of the extract was assessed, and its ability to induce apoptosis was examined. Additionally, the effects of TCE on colony formation and cell migration were investigated. The findings from the present study provide further insights into the therapeutic potential of *T. chantrieri* as a natural alternative for breast cancer treatment.

Materials and methods

Plant materials and extraction

Fresh rhizomes of *T. chantrieri* purchased from a local cultivator in Chiang Rai Province, Thailand, were taxonomically authenticated by Dr. Chaiyong Rujjanawate as identical to voucher specimen No. 168-M, which has been deposited at the School of Medicine Herbarium, Mae Fah Luang University, Thailand. Fresh rhizomes were thoroughly cleaned, coarsely chopped, shade-dried, and ground into a coarse powder. The powdered rhizome was then subjected to extraction with 95% ethanol, and the solvent was subsequently removed under reduced pressure using a rotary evaporator. After ethanol removal, the dried crude extract was dissolved in distilled water and subjected to liquid-liquid partitioning. The aqueous solution was extracted 3 times with an equal volume of hexane, and the hexane layers were discarded. The remaining aqueous phase was extracted 3 times with dichloromethane, and those fractions were also discarded. Finally, the residual aqueous layer was extracted 3 times with n-butanol. The combined n-butanol extracts were evaporated under reduced pressure at 40 °C and then lyophilized to yield the final n-butanol-soluble fraction for subsequent assays. This 3-solvent extraction protocol was adapted from a previously established method that effectively separates plant constituents by polarity [8]. The extraction process is presented in **Figure 1**. The lyophilized extract was reconstituted in deionized water and filtered through a 0.2 μm syringe filter before cell treatment. A schematic diagram of the experimental design is illustrated in **Figure 2**.

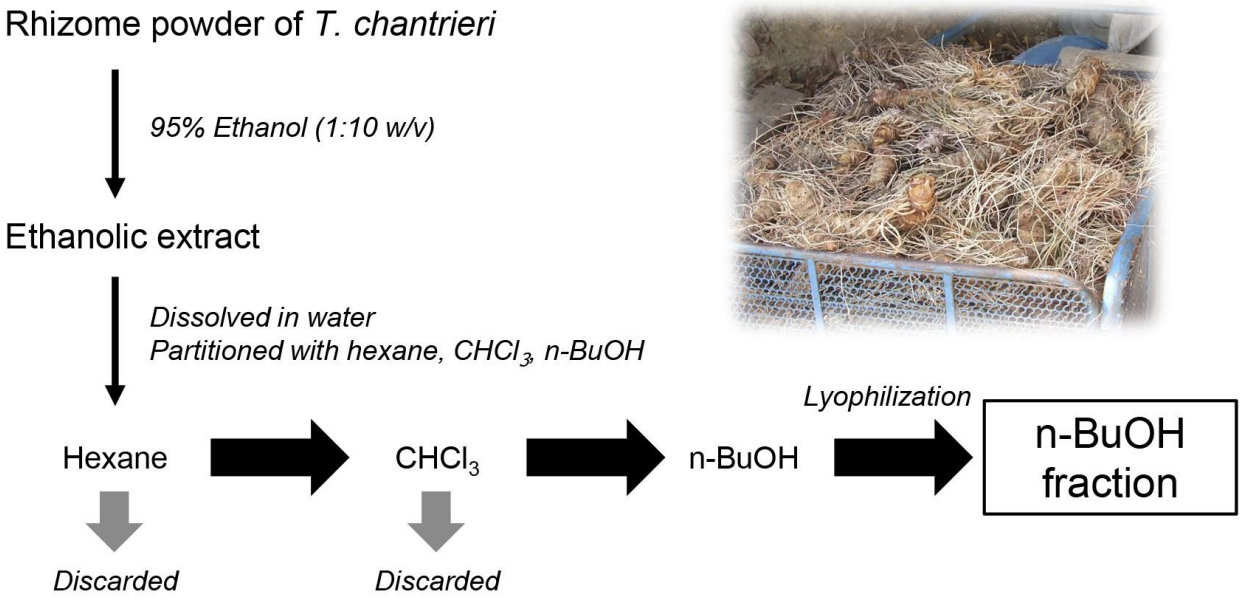

Figure 1 A schematic diagram representing the extraction protocol. The dried crude extract was redissolved in distilled water (1:10 w/v) and partitioned 3 times with hexane and dichloromethane (both phases discarded), followed by 3 extractions with n-butanol. The n-butanol layer was concentrated under reduced pressure at 40°C and lyophilized to yield the bioactive n-butanol fraction.

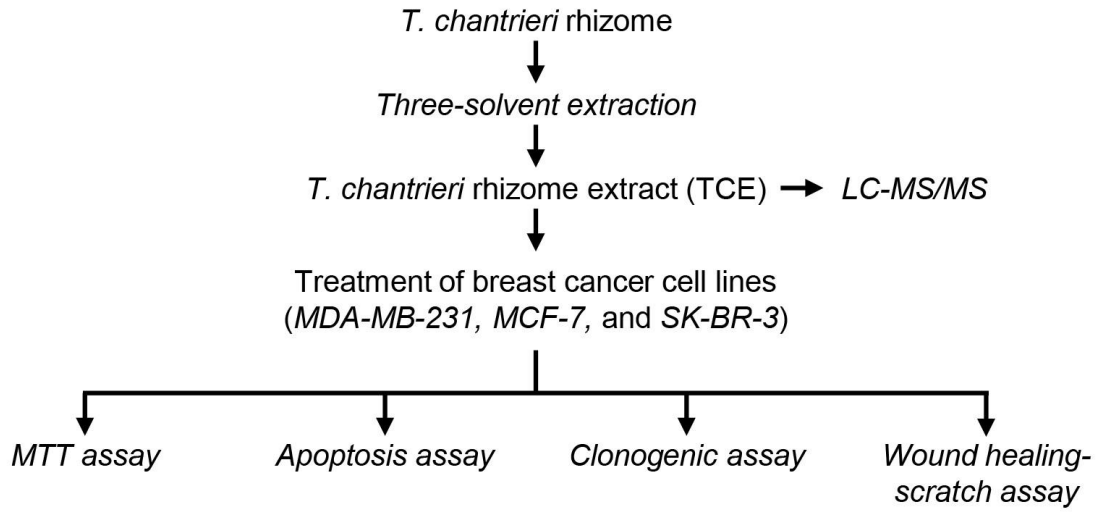

Figure 2 A schematic diagram of the experimental design.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of TCE

The TCE (1 mg/mL in methanol; 0.5 µL injection) was separated on a Poroshell EC-C18 column (2.7 µm, 2.1×150 mm^2) with a binary mobile phase of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at 0.2 mL/min, and data were acquired using Agilent MassHunter. Mass spectrometry (Agilent Q-TOF MS) employed a gas temperature of 300 °C, gas flow of 10 L/min, nebulizer pressure of 40 psi, sheath gas flow of 12 L/min, capillary voltage of 3,500 V, nozzle voltage of 3.5 kV, and draw/ejection speeds of 100/400 µL/min. Spectra (50 - 1,100 amu) were recorded at 4 spectra/s in MS and automatic MS/MS modes using collision energies of 10, 20, and 40 eV.

Cell culture

Human breast cancer cell lines, including MDA-MB-231 (triple negative; HTB-26™), MCF-7 (ER-positive; HTB-22™), SK-BR-3 (HER2-positive; HTB-30™), and normal breast epithelial MCF-10A cells (CRL-10317™), were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco/Invitrogen Life Technologies, Carlsbad, CA, USA) with 10% (v/v) fetal bovine serum (FBS; Gibco), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco) at 37 °C with 5% CO_2.

MTT assay

The MDA-MB-231, MCF-7, SK-BR-3, and MCF-10A cells (5×10^3 cells/well) were seeded into a 96-well plate and incubated overnight. The cells were then treated with varying concentrations of TCE (3.12 - 200 µg/mL) for 24 h. Following treatment, the culture medium was discarded, and the cells were incubated with 0.5 mg/mL of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) for 2 h at 37 °C. After incubation, the supernatant was removed, and dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals. The optical density (OD) at 570 nm was recorded using a Tecan Spark multimode microplate reader (Tecan Austria GmbH, Grödig, Austria). Cell viability was expressed as a percentage relative to untreated control cells. The half-maximal inhibitory concentration (IC_{50}) was determined using GraphPad Prism (version 8.0.1; GraphPad Software, San Diego, CA, USA).

Apoptosis assay

The percentage of apoptotic cells was determined using annexin V and propidium iodide (PI) staining with the Annexin V-FITC Apoptosis Staining/Detection Kit (ab14085; Abcam, Cambridge, UK) as previously described [15,16]. MDA-MB-231, MCF-7, and SK-BR-3 cells (1.8×10^5 cells/well) were seeded into a 12-well plate and incubated overnight. The cells were treated with TCE (12.5 - 100 µg/mL) for 24 h. After treatment, the cells were harvested using TrypLE Express reagent

(Gibco) and stained with annexin V-FITC and PI solution. Flow cytometry was performed using a DxFLEX flow cytometer (Beckman Coulter Inc., IN, USA), and the data were analyzed with CytExpert for DxFLEX software (version 2.0.0.283; Beckman Coulter Inc.).

Clonogenic assay

A clonogenic assay was performed as previously described [17]. MDA-MB-231, MCF-7, and SK-BR-3 cells (5×10^3 cells/well) were seeded into a 96-well plate and incubated overnight. The cells were treated with TCE (12.5 - 100 µg/mL) for 24 h. After treatment, 1×10^3 cells were transferred to a 12-well plate and cultured in a complete medium for 5 - 7 days, with the medium refreshed every 2 days. For colony visualization, the cells were fixed with methanol for 15 min and stained with 0.5% (w/v) crystal violet overnight. After washing, stained cell colonies were imaged using an inverted microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). The colonies were then dissolved in 33% (v/v) acetic acid, and absorbance at 590 nm was measured using a Tecan Spark multimode microplate reader (Tecan Austria GmbH).

Wound healing-scratch assay

Cell migration was assessed using a wound healing-scratch assay, as previously described [18]. MDA-MB-231, MCF-7, and SK-BR-3 cells (1.8×10^5 cells/well) were seeded into a 12-well plate and incubated overnight. Once the cells reached 100% confluence, a scratch was created using a 200-µL pipette tip. The adherent cells were washed with PBS and then treated with TCE (12.5 - 100 µg/mL) for 24 h. Images of the wound area were captured before treatment (0 h) and after 24 h using an inverted microscope (Carl Zeiss Microscopy GmbH). The wound area was measured in at least 5 random fields per sample using ImageJ software (version 1.54d) with the wound healing size tool plugin [19]. The percentage of wound closure was calculated using the formula: Percentage of wound closure = (Wound area at 0 h − Wound area after 24 h)/Wound area at 0 h×100.

Statistical analysis

All statistical analyses were performed using GraphPad Prism (version 8.0.1; GraphPad Software). Results are presented as the mean ± SD from 3 replicates, and differences were compared using 1-way ANOVA followed by Tukey's post hoc test. A *p*-value of less than 0.05 was considered statistically significant.

Results and discussion

Effect of TCE on the viability of breast cancer cell lines

The rhizomes of *T. chantrieri* were extracted using the described method, and their phytochemical composition is summarized in **Table 1**. To evaluate its cytotoxic effects, TCE was tested on normal breast epithelial MCF-10A cells and 3 genetically distinct breast cancer cell lines: MDA-MB-231, MCF-7, and SK-BR-3. After 24-hour treatment, results from the MTT assay revealed that TCE did not exhibit severe cytotoxic effects on normal breast epithelial MCF-10A cells (IC_{50} > 200 µg/mL). The IC_{50} values for each cell line are summarized in **Table 2**. In contrast, a dose-dependent reduction in cell viability was observed in MDA-MB-231 (**Figure 3(A)**), MCF-7 (**Figure 3(B)**), and SK-BR-3 (**Figure 3(C)**) cells following treatment with TCE. These findings suggest that TCE selectively exhibits cytotoxicity against breast cancer cells at low concentrations. Moreover, these results are consistent with a previous study demonstrating that TCE at concentrations of 10 - 12.5 µg/mL significantly reduces the viability of cholangiocarcinoma cells [14]. This evidence supports the potential of TCE as an anti-cancer agent against multiple cancer types.

Table 1 Phytochemicals identified from TCE and their reported anticancer activity.

Compound name	Retention time (min)	m/z	Intensity (Area)	Formula	Anti-cancer activity	Ref.(s)
Gibberellin A4 glucosyl ester	16.146	493.208	50,832,196	$C_{25}H_{34}O_{10}$	Induces apoptosis in A549 lung cancer cells via mitochondrial and ER stress pathways	[20]
4-hydroxyandrostenedione glucuronide	18.766	523.2186	44,964,812	$C_{25}H_{34}O_{9}$	Suppresses estradiol levels in postmenopausal breast cancer patients	[21]
3-epigibberellin A1	14.394	347.1499	10,665,814	$C_{19}H_{24}O_{6}$	Induces G2/M phase arrest and apoptosis in MCF-7 breast cancer cells	[22]
Isopulegone caffeate	20.029	315.160	7,747,607	$C_{19}H_{24}O_{4}$	Decreases cell viability and increases apoptosis in A549 lung cancer cells	[23]
Angiotensinamide	28.309	1029.5275	7,719,296	$C_{49}H_{70}N_{14}O_{11}$	-	
Soyasaponin A5	28.308	1075.5329	5,777,465	$C_{52}H_{84}O_{23}$	Induces apoptosis in gastric cancer cells	[24]
Chantriolide B	16.461	811.3397	6,122,506	$C_{38}H_{54}O_{16}$	-	
Pregna-5,16-dien-3β-ol-20-one 3-O-β-chacotrioside	27.204	813.3912	3,921,256	$C_{39}H_{60}O_{15}$	-	
1α,3β,22R-Trihydroxyergosta-5,24E-dien-26-oic acid glycoside	26.610	945.4702	3,555,915	$C_{46}H_{74}O_{20}$	-	
Trigofoenoside D	25.903	1063.5332	4,759,548	$C_{51}H_{84}O_{23}$	-	
Dai-tunicamine	10.772	431.1925	4,534,706	$C_{20}H_{32}O_{10}$	Reduces cell growth and enhances apoptosis in breast (MCF-7, SKBR-3) and colon (HCT116, HT-29) cancer cells	[25,26]
Chantriolide A	16.010	809.3239	4,645,403	$C_{38}H_{52}O_{16}$	-	
(7'R,8'R)-4,7'-Epoxy-3'-methoxy-4',5,9,9'-lignanetetrol 9'-glucoside	12.194	507.1875	4,532,192	$C_{25}H_{32}O_{11}$	-	
(2R)-1-O-β-D-Galactopyranosylglycerol	1.705	253.0929	3,170,139	$C_{9}H_{18}O_{8}$	-	
Alectrol	14.104	345.1346	2,675,566	$C_{19}H_{22}O_{6}$	-	
Dimethylbenzyl carbinyl hexanoate	27.409	293.1759	1,257,573	$C_{16}H_{24}O_{2}$	-	
Isopalmitic acid	31.900	255.2327	1,553,721	$C_{16}H_{32}O_{2}$	-	
Gibberellin A37 glucosyl ester	19.454	553.2294	3,341,881	$C_{26}H_{36}O_{10}$	-	
Gibberellin A38 glucosyl ester	16.578	523.2187	1,996,954	$C_{26}H_{36}O_{11}$	-	

Table 2 The IC_{50} values of TCE for each cell line.

Cell line	IC_{50} (μg/mL)[#]
MCF-10A	> 200
MDA-MB-231	15.09 ± 0.10
MCF-7	15.87 ± 2.51
SK-BR-3	32.11 ± 1.60

[#] Data represent mean ± SD (N = 3)

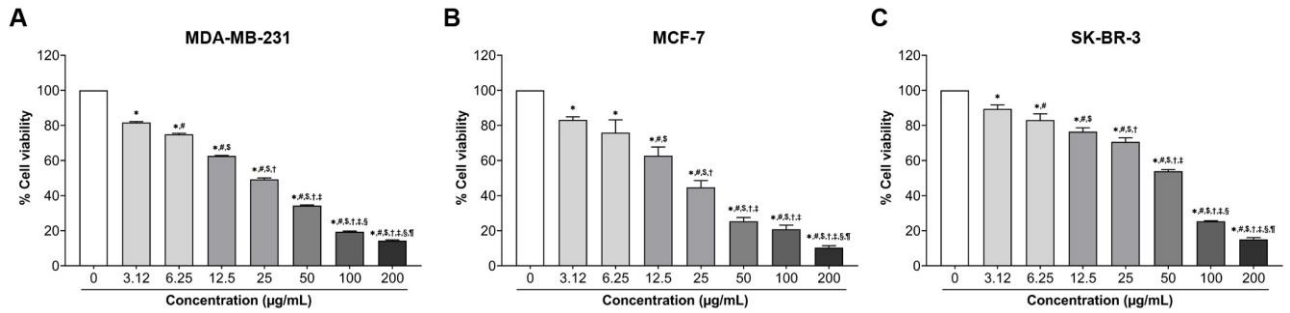

Figure 3 Cell viability of breast cancer cells after treatment with TCE for 24 h. The MTT assay was conducted on MDA-MB-231 (A), MCF-7 (B), and SK-BR-3 (C) cells after 24-hour treatment with TCE. Data are presented as the mean ± SD (N = 3). * $p < 0.05$ vs. the untreated control (0 μg/mL), $^{\#}$ $p < 0.05$ vs. 3.12 μg/mL, $^{\$}$ $p < 0.05$ vs. 6.25 μg/mL, † $p < 0.05$ vs. 12.5 μg/mL, ‡ $p < 0.05$ vs. 25 μg/mL, § $p < 0.05$ vs. 50 μg/mL, ¶ $p < 0.05$ vs. 100 μg/mL.

Effect of TCE on apoptosis of breast cancer cell lines

Annexin V and PI staining were performed to determine the percentage of apoptotic cells in breast cancer cell lines following 24 h of treatment with TCE. The results showed that TCE dose-dependently induced apoptosis in all breast cancer cells (**Figure 4**). These findings suggest that TCE exerts broad-spectrum anti-cancer activity against genetically distinct breast cancer cells. In addition, an increased number of early apoptotic cells was observed in all breast cancer cell lines, particularly in triple-negative breast cancer (TNBC) MDA-MB-231 cells, highlighting its potential therapeutic relevance for targeting this difficult-to-treat cancer subtype. Apoptosis is a form of programmed cell death, and its dysregulation contributes to cancer progression [27]. Natural compounds that promote apoptosis have gained interest as potential therapeutic agents for breast cancer treatment [28]. A previous study has shown that TCE affects the Bcl-2/Bax apoptosis pathway in cholangiocarcinoma cells [14]. Several bioactive compounds in *T. chantrieri* have been reported to induce apoptosis in cancer cells. For instance, gibberellin and caffeate derivatives induce apoptosis in lung cancer cells via mitochondrial pathways [20,23]. A bioactive spirostanol saponin has been shown to promote apoptosis in gastric cancer cells by regulating the c-Jun N-terminal kinase (JNK) pathway [29]. Taccalonolides affect tubulin dynamics, leading to mitotic spindle defects and apoptosis [30,31]. Withanolides trigger mitochondrial dysfunction to activate apoptosis in TNBC [32]. Soyasaponin Ag promotes TNBC cell apoptosis and suppresses tumor growth via inactivating the dual specificity phosphatase 6 (DUSP6)/mitogen-activated protein kinase (MAPK) pathway [33]. Apoptosis is regulated by multiple factors and involves cross-talk with various signaling pathways [34]. Thus, the anti-cancer activity of TCE may be mediated by several bioactive compounds acting on different apoptosis-related molecules, particularly components of the intrinsic apoptotic pathway. However, further analysis of molecular markers is needed to identify the specific pathways and key targets involved.

Effect of TCE on colony formation of breast cancer cell lines

Colony formation is a key characteristic of cancer cells that contributes to tumor development and metastasis [35,36]. In this study, a clonogenic assay was performed to assess the effect of TCE on the colony-forming ability of breast cancer cells. As shown in **Figure 5**, TCE treatment dose-dependently inhibited colony formation in MDA-MB-231, MCF-7, and SK-BR-3 cells, suggesting its potential to suppress long-term cancer cell proliferation. The inhibition of colony formation is associated with cell cycle arrest and various signaling pathways [37-39]. Although the exact bioactive compounds and underlying mechanisms are still unknown, these findings support TCE as a promising anti-cancer agent that effectively suppresses the long-term growth of breast cancer cells.

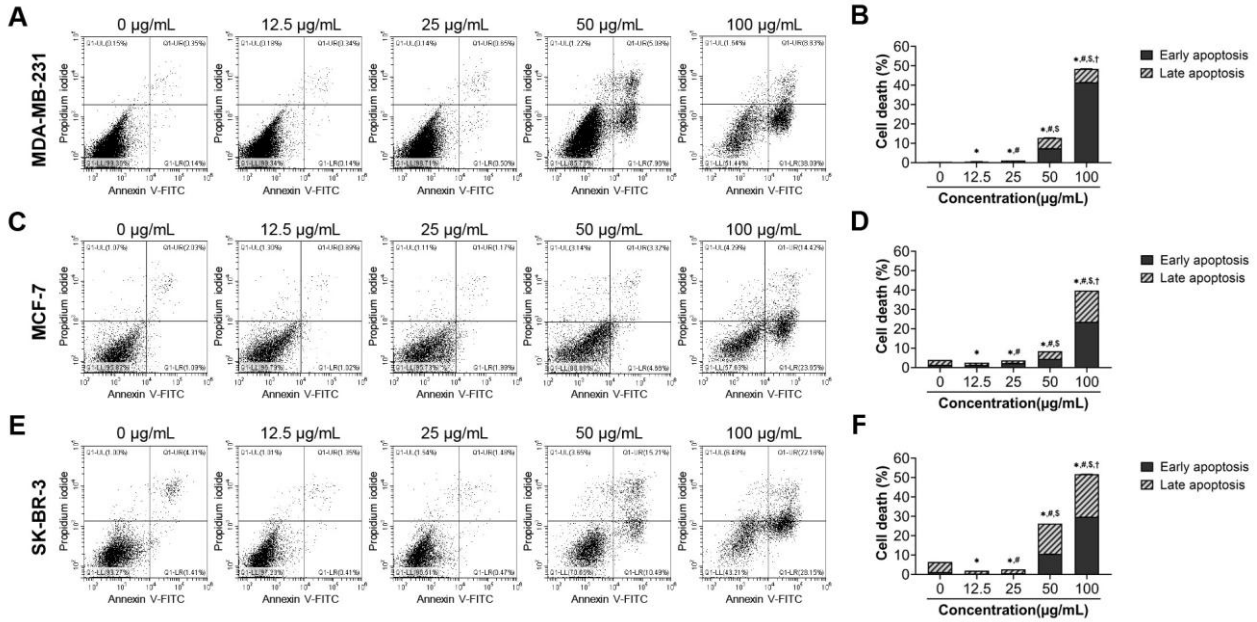

Figure 4 The percentage of apoptotic breast cancer cells after treatment with TCE for 24 h. Annexin V and PI staining were performed on MDA-MB-231, MCF-7, and SK-BR-3 cells after 24 h of TCE treatment. Representative flow cytometry dot plots of annexin V and PI-stained cells in MDA-MB-231 (A), MCF-7 (C), and SK-BR-3 (E). The percentage of apoptotic cells in MDA-MB-231 (B), MCF-7 (D), and SK-BR-3 (F). Data are expressed as mean ± SD (N = 3). * $p < 0.05$ vs. the untreated control (0 µg/mL), $^{\#}$ $p < 0.05$ vs. 12.5 µg/mL, $^{\$}$ $p < 0.05$ vs. 25 µg/mL, † $p < 0.05$ vs. 50 µg/mL.

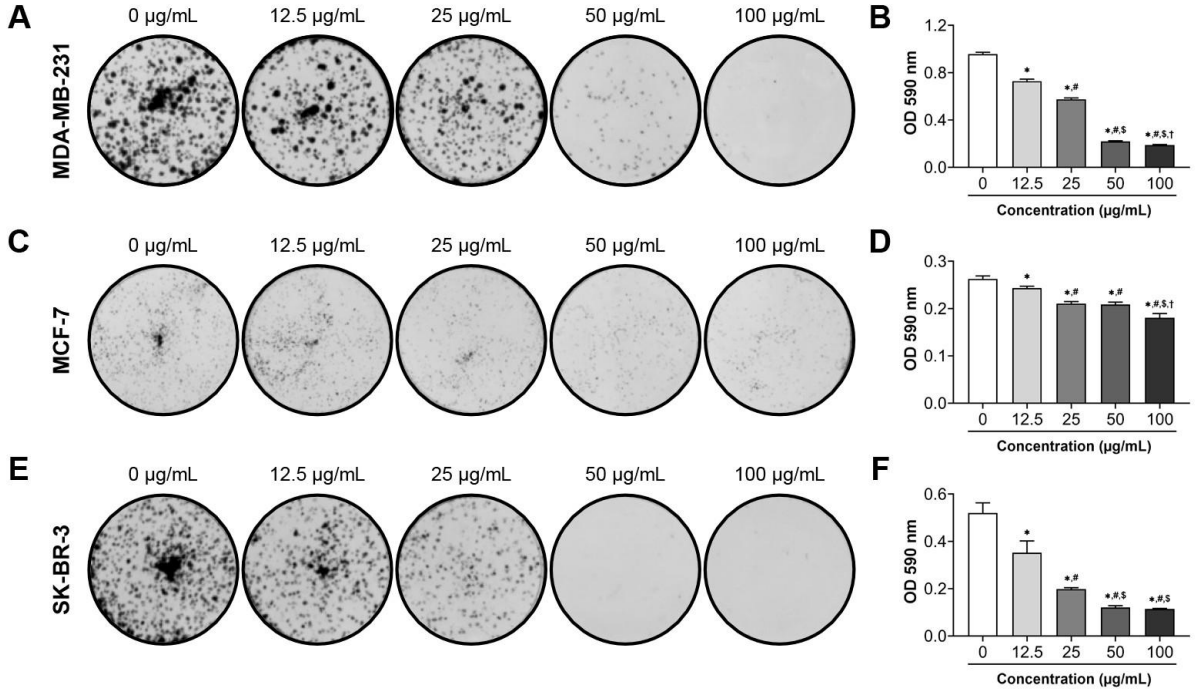

Figure 5 Colony formation of breast cancer cells after treatment with TCE for 24 h. A clonogenic assay was performed on MDA-MB-231, MCF-7, and SK-BR-3 cells after 24 h of TCE treatment. Representative images of formed colonies in MDA-MB-231 (A), MCF-7 (C), and SK-BR-3 (E). The absorbance at 590 nm of crystal violet-stained colonies in MDA-MB-231 (B), MCF-7 (D), and SK-BR-3 (F). Data are expressed as mean ± SD (N = 3). * $p < 0.05$ vs. the untreated control (0 µg/mL), $^{\#}$ $p < 0.05$ vs. 12.5 µg/mL, $^{\$}$ $p < 0.05$ vs. 25 µg/mL, † $p < 0.05$ vs. 50 µg/mL.

Effect of TCE on the migration of breast cancer cell lines

Cancer cell migration is a critical step in tumor progression and metastasis [40]. To evaluate the effect of TCE on breast cancer cell migration, a wound healing-scratch assay was performed. The results showed that TCE dose-dependently inhibited the migration of all breast cancer cells (**Figure 6**). Notably, the highly invasive TNBC cell line MDA-MB-231 exhibited a pronounced reduction in migration. Many natural compounds exert their anti-cancer effects by suppressing cancer cell migration [3]. For example, the spirostanol saponin timosaponin AIII (TA3) has been shown to suppress human osteosarcoma cell migration by inhibiting matrix metalloproteinases (MMPs) [41].

Additionally, withanone and withaferin A, the major withanolides, inhibit cell migration by targeting heterogeneous nuclear ribonucleoprotein K (hnRNP-K) and its downstream effectors [42]. These findings suggest that TCE effectively inhibits the migration of breast cancer cells. This effect may involve modulation of cytoskeletal dynamics and suppression of MMP activity. Since targeting cancer cell migration and invasion is a promising therapeutic strategy [43], the observed inhibitory effects highlight the potential of TCE as a natural agent to suppress breast cancer progression.

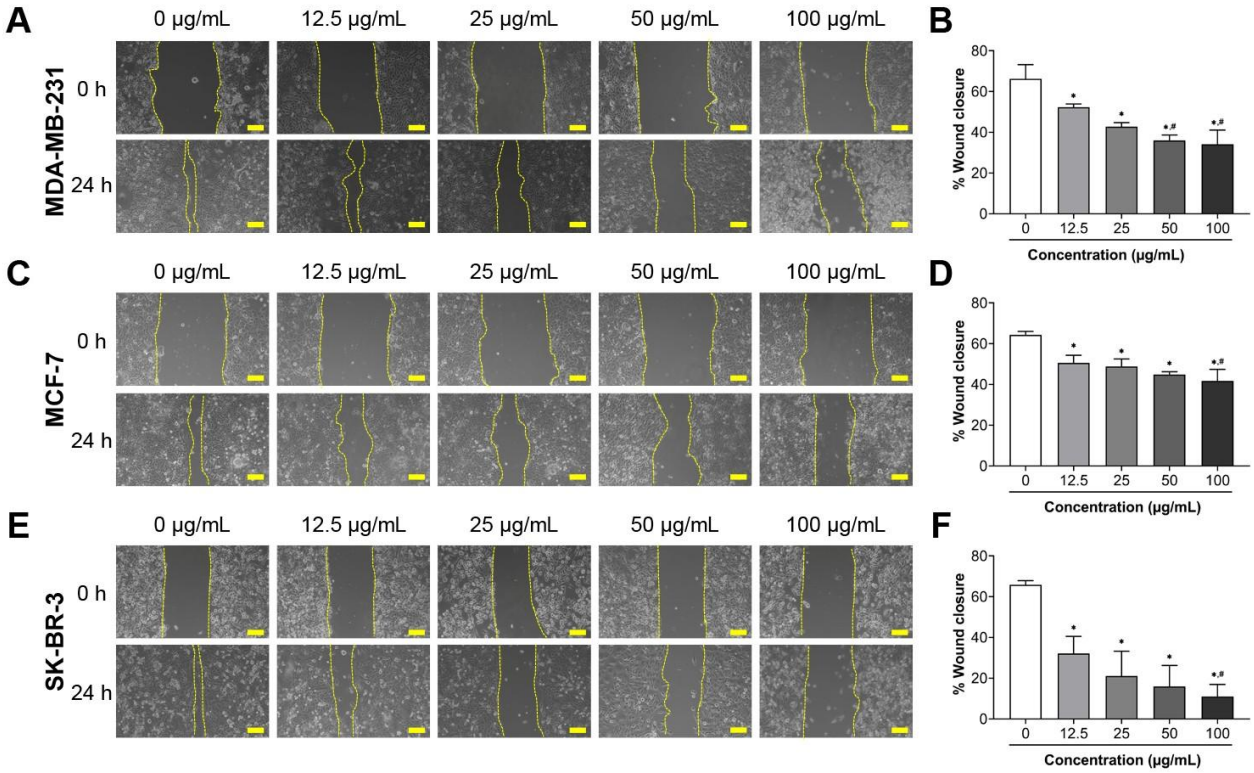

Figure 6 Migration of breast cancer cells after treatment with TCE for 24 h. A wound healing-scratch assay was conducted on MDA-MB-231, MCF-7, and SK-BR-3 cells after 24 h of TCE treatment. Representative images of the wound area before treatment (0 h) and after 24 h of treatment in MDA-MB-231 (A), MCF-7 (C), and SK-BR-3 (E). Scale bar = 100 μm. The percentage of wound closure in MDA-MB-231 (B), MCF-7 (D), and SK-BR-3 (F). Data are expressed as mean ± SD (N = 3). * $p < 0.05$ vs. the untreated control (0 μg/mL), $^{\#}$ $p < 0.05$ vs. 12.5 μg/mL.

Despite these promising findings, the present study has some limitations. The exact molecular mechanisms underlying its anticancer effects are still unclear and require further investigation. Additionally, the bioavailability, pharmacokinetics, and potential toxicity of *T. chantrieri* need to be assessed in animal and clinical studies to confirm its safety and effectiveness. Investigating combination therapies could further support the clinical application of *T. chantrieri* in cancer treatment.

To fully understand the therapeutic potential of *T. chantrieri*, it is important to identify and isolate the bioactive compounds responsible for its anti-cancer activity. A bioactivity-guided fractionation approach, combined with chromatographic techniques such as high-performance liquid chromatography (HPLC) and LC-MS, can be employed to isolate active compounds. Subsequent structural elucidation, *in vitro* bioassays, and omics-based analyses would help determine the molecular targets and mechanisms of action of these compounds. Together, these strategies may lead to the identification of lead compounds and support the future development of *T. chantrieri* as an alternative cancer therapy.

Conclusions

The present study demonstrated that *T. chantrieri* exhibits promising anti-cancer activity against breast cancer cell lines. TCE showed selective cytotoxicity toward cancer cells, with minimal effects on normal breast epithelial MCF-10A cells. It significantly reduced cell viability, induced apoptosis, and inhibited colony formation and migration in MDA-MB-231, MCF-7, and SK-BR-3 cells (**Figure 7**). These findings provide valuable insights into the potential of *T. chantrieri* as a natural source of bioactive compounds for the development of alternative therapies for breast cancer. Further studies are warranted to investigate its underlying mechanisms and evaluate its efficacy in animal models and clinical settings.

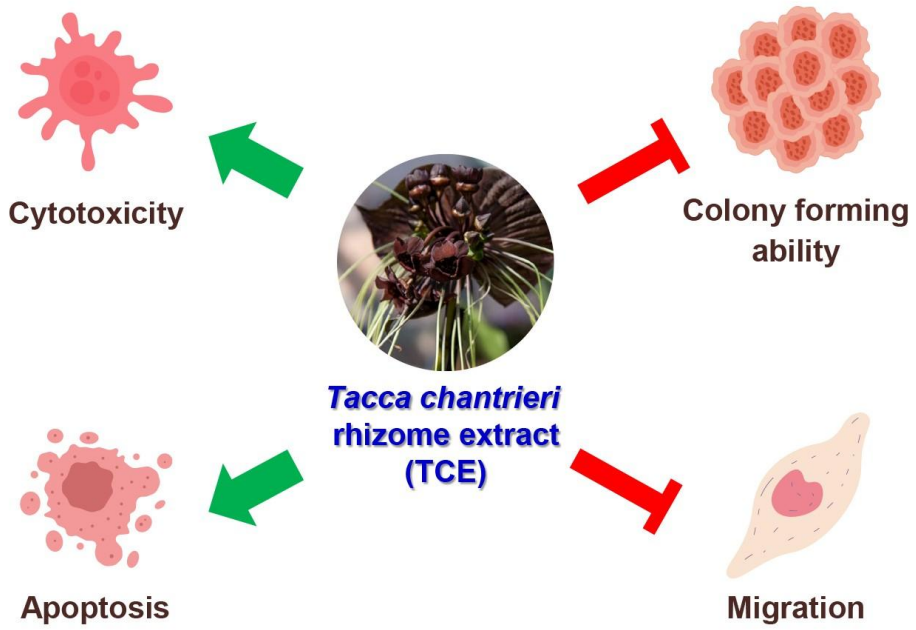

Figure 7 Anti-cancer effects of TCE against breast cancer cell lines.

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Declaration of Generative AI in Scientific Writing

During the preparation of this work the authors used ChatGPT in order to verify the grammar and improve the readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT Author Statement

Sirinda Thewaanukhor: Investigation, Formal analysis, Writing - Original Draft. **Artitaya Rongjumnong**: Investigation, Formal analysis. **Papawarin Karamart**: Investigation, Formal analysis. **Orrapan Vito**: Investigation, Formal analysis. **Siripat Aluksanasuwan**: Conceptualization, Formal analysis, Visualization, Writing - Review & Editing. **Narudol**

Teerapattarakan: Investigation, Formal analysis. **Atthapan Morchang**: Conceptualization, Formal analysis. **Phateep Hankittichai**: Conceptualization, Formal analysis. **Chutamas Thepmalee**: Formal analysis, Supervision. **Keerakarn Somsuan**: Conceptualization, Investigation, Formal analysis, Visualization, Funding acquisition, Writing - Review & Editing.

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