https://doi.org/10.48048/tis.2024.8125

# Pinostrobin Exerts Inhibitory Effects on Adipogenesis and Adipocyte-Induced MCF-7 Breast Cancer Cell Proliferation and Migration

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*Received: 27 February 2024, Revised: 29 March 2024, Accepted: 7 April 2024, Published: 30 July 2024*

**Abstract**

Dysfunctional adipose tissue contributes to several diseases, including diabetes, cardiovascular disease, and some cancers. Among the global female population, breast cancer is a cancer type that is increasing gradually in annual cases, while it also has the highest mortality rate. Recent evidence indicates that accumulation of the adipose tissue mass contributes to the progression of breast cancer proliferation and metastasis. The purposes of this study are to investigate the effects of pinostrobin - a compound extracted from *Boesenbergia rotunda* (*L.*) - on 3T3-L1 preadipocyte differentiation and migration as well as its inhibitory effects on adipocyte-induced MCF-7 proliferation and migration. This study revealed that pinostrobin at a concentration of 0 to 25 µM was not toxic to the 3T3-L1 cells and treatment with 25 µM pinostrobin dramatically reduced the adipogenesis of 3T3-L1 compared to untreated cells, as measured by both Oil Red O and Nile red fluorescence staining. In addition, the migratory ability of 3T3-L1 was reduced at 6 h post-treatment measured by wound healing migration assay. An indirect co-culture system of MCF-7 and an adipocyte-conditioned medium revealed a further increase in the ability of the MCF-7 to migrate and proliferate. Interestingly, treatment with pinostrobin at a concentration of 25 µM could suppress cell migration and inhibit the colony formation of MCF-7 breast cancer cells in the context of this co-culture system. Based on the findings of this study, pinostrobin could be a promising therapeutic candidate as a novel cancer treatment drug used in patients with obesity-related breast cancer in the future.

**Keywords:** Breast cancer, Pinostrobin, Adipogenesis, *Boesenbergia rotunda* (*L.*), Adipocytes

## Introduction

Nowadays, breast cancer is one of the highest new cases and mortality rate type of cancer in women worldwide reported by the World Health Organization (WHO). Several risk factors such as age, hormonal alteration, radiation exposure, consumption of carcinogenic-containing products, hereditary causes, and also metabolic reprogramming have been known to correlate with an increase in the developmental rate of breast cancer [1-3].

Fundamentally, adipocytes and adipose tissue are derived from the differentiation of precursor cells (preadipocytes) [4,5]. During adipogenesis, the activation of two critical transcription factors - Peroxisome proliferator-activated receptor gamma and CCAAT/enhancer-binding protein alpha - followed by other adipogenic markers expression, is a critical mechanism [4-9]. In addition, the enhancement of preadipocyte migration from the original location into fat cell clusters is one of the crucial steps in adipocyte development and adipocyte hyperplasia [10]. Adipose tissue is not only an important organ responsible for several functions in the body but also plays a role in the progression of breast cancer cells. The metabolic reprogramming and the alteration of tumor microenvironments, such as the accumulation of tumor-surrounding adipose tissue as well as improper adipocyte functions, are associated with increasing the risk of the development and promoting metastasis of several types of breast cancers particularly estrogen receptor-positive [3,11-13]. The intra-tumoral adipocytes have been known to interact with cancer cells and aggravate cancer progression by stimulating the release of TNF-α, IL-6, sphingosine-1-phosphate, etc. [14,15]. Additionally, a high amount of intra-tumoral adipocyte and the increase of its migratory ability are not only associated with inflammation and metastatic pathways but also with a decrease in survival [10,16].

Nowadays, several methods are used to treat breast cancer patients. However, such treatments are still limited and have many side effects [17]. Thus, there is still a need for new treatment options. Medicinal compounds extracted from herbs, fruits, or vegetables have lately drawn considerable attention for their potential use as novel drug candidates for treating various diseases, including cancer, due to their diverse pharmacological effects [18-22]. Pinostrobin (2,3-dihydrotectochrysin), the active substance of the flavanone class, exerts several pharmacological activities [23-26]. In the context of the anti-cancer property, until recently, no known studies have focused on the inhibitory effects of this compound on the proliferation and migration of breast cancer cells in adipocyte-induced conditions. To fill this knowledge gap, the effects of pinostrobin on adipogenesis as well as the effects of pinostrobin on adipocyte-induced breast cancer cell proliferation and migration are investigated.

## Materials and methods

### Chemicals and reagents

Pinostrobin (purity > 98 % by HPLC) was isolated from *Boesenbergia rotunda* (*L.*). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, 0.25 % trypsin-EDTA, and Penicillin-Streptomycin were purchased from Gibco (Waltham, MA, USA). Crystal-violet, isobutyl-3-methylxanthine (IBMX), dexamethasone (DEX), insulin, Nile red fluorescence, and Oil Red O were purchased from Sigma-Aldrich

(St. Louis, MO, USA). The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Medchem Express (Monmouth Junction, NJ, USA). All other additional chemicals used in this study were analytical grade.

**Cell culture**

3T3-L1, a fibroblast cell isolated from the embryo of a mouse, and MCF-7, a human adenocarcinoma with high expression of estrogen receptor-positive (ER+), were purchased from the American Type Culture Collection (Manassas, VA, USA). The 3T3-L1 cell was used as a model in this study because it is a well-known *in vitro* model used for adipose tissue-related studies due to it being easier to maintain and less costly, while also producing a more homogenous response to the treatment than other models [27] whereas the MCF-7 was used as a representative for ER+ breast cancer cell model because this type of cancer can be found in approximately 70 % of all breast cancer cases in the world [28]. For both cell types, cells were cultured in Dulbecco's modified Eagle's medium high glucose supplemented with 10 % fetal bovine serum (FBS) and 1 % of penicillin/streptomycin. The cells were cultured in a humidified incubator under appropriate conditions (5 % $CO_2$ at 37 °C and 95 % air atmosphere). Once confluent, the cells were washed with phosphate buffer saline (PBS) and sub-cultivated by 0.25 % Trypsin EDTA. Before performing the co-culture experiments, it was necessary for the 3T3-L1 cells to differentiate into mature adipocytes. Thus, when 3T3-L1 reached 100 % confluency, the cells were cultured for another 48 h in order to stimulate contact inhibition and they were then activated with 1 µM dexamethasone (DEX), 10 µg/ml insulin and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) for an additional 48 h. After induction, the cells were differentiated from preadipocytes into mature adipocytes. The period from stimulation to becoming adult fat cells was approximately 10 days.

**Condition medium preparation**

The 3T3-L1 cells were cultured and grown until 100 % confluency was achieved. The cells were then cultured for another 48 h to achieve contact inhibition and then differentiated with adipogenic cocktails (1µM dexamethasone, 10 µg/mL insulin and 0.5 mM IBMX) for an additional 48 h. Next, the cells were placed in an insulin medium for another 48 h and were cultured until day 10. A culture medium derived from mature adipocytes at day 10 was used for indirect co-culture experiments.

**Cell cytotoxicity assay using crystal violet staining and MTT assay**

The 3T3-L1 cells at a concentration of $1\times10^4$ cells/well were placed in a 96-well plate. After 24 h of cultivation, the cells were then treated with various concentrations of pinostrobin at concentrations of 0, 5, 10, 25 and 50 µM. For the crystal violet staining assay, after 48 h of treatment, the cells were then washed with PBS and fixed with 10 % formaldehyde for 30 min. Next, the cells were washed twice with PBS and stained with 0.05 % crystal violet for 30 min. Then, the cells were rinsed with water twice and air dried thoroughly, before 100 µL of methanol was added to every well and incubated for 30 min allowing the crystal violet dye to dissolve. Finally, the absorbance was then measured by a microplate reader at 590 nm. The percentage of cell survival was calculated as % cell viability using the following equation:

$$\%\ cell\ viability = \frac{\text{ODsample} - \text{ODblank}}{\text{ODcontrol} - \text{ODblank}} \times 100$$

In terms of the MTT assay, the cells were treated with pinostrobin at various concentrations for 48 and 72 h. Next, 0.5 mg/mL of MTT reagent was added and was then incubated for another 4 h in the dark. The 100 µl of DMSO was added and used as a solubilized reagent to dissolve the purple formazan crystals. The absorbance was measured at a wavelength of 570 nm using a microplate reader. The percentage of cell viability was calculated using the following equation:

$$\%\ cell\ viability = \frac{\text{ODsample} - \text{ODblank}}{\text{ODcontrol} - \text{ODblank}} \times 100$$

**Oil red O staining (ORO)**

To investigate the accumulation of neutral lipids in the 3T3-L1 cells, Oil Red O staining was used. The 3T3-L1 cells were cultured and induced to differentiate into mature adipocytes by using adipogenic cocktails in the presence or absence of pinostrobin for 10 days. On day 10, the cells were fixed with 10 % formaldehyde for 20 min, washed with PBS twice, and allowed to air dry. Next, the cells were stained with a working solution of Oil Red O for 60 min and rinsed with $dH_2O$ 4 times. The stained cells were then observed and pictured under a light microscope. The intensity derived from Oil Red O stained cells was obtained from the incubation of stained cells in 100 % isopropanol for 30 min and measured by a microplate reader at 500 nm.

**Nile red and DAPI staining**

For fluorescence staining, the post-treated cells were fixed with 4 % formaldehyde for 30 min. The intracellular neutral lipid within the cells was then stained with 1 µg/mL Nile red at 37 °C in the dark for 10 min. Next, the nucleases of the cells were co-stained with 1 µg/mL DAPI at 37 °C in the dark for another 10 min. Finally, fluorescence imaging was performed under a fluorescence microscope and the fluorescence intensity obtained from the images was analyzed using Image J Software. The data were presented as average intensities obtained from at least five independent fields per well under a 10× objective lens.

**Wound healing migration assay**

The effects of pinostrobin on migratory ability were investigated using an indirect co-culture system of 3T3-L1 and MCF-7 cells. In this study, three experimental groups were divided: MCF-7 cultured in a complete medium (Control), MCF-7 cultured in a conditioned medium derived from mature fat cells (CM), and MCF-7 cultured in a conditioned medium with pinostrobin added (CM+ pi). The MCF-7 cells were cultured in a 6-well plate until they reached 100 % confluency. A ratio of 70 % CM was then placed in a cultured plate, either alone (CM condition) or in combination with pinostrobin (CM + pi). The monolayer cells were scratched in each well with a sterile 200 µL tip. The width of the scratches in each group at 6 h

was captured under a light microscope and compared with the width in each group at 0 h. The percentage of the remaining area of width was then calculated and represented as a percentage of control.

$$Scratch \text{ closer} = \frac{\text{At0} - \text{At}}{\text{At0}} \times 100$$

where: $A_{t0}$ and $A_t$ represent the scratch area at 0 and 6 h, respectively.

**Colony formation assay**

The concentration of $1.5\times10^3$ cells per well of MCF-7 were cultured in a 6-well plate for 24 h. The cells were then treated with various conditions as follows: MCF-7 cultured in complete medium (Control), MCF-7 cultured in condition medium derived from mature fat cells (CM), and MCF-7 cultured in condition medium derived from mature fat cells with pinostrobin added (CM+ pi) for 16 days with the medium and compound changed every three consecutive days. At day 16, the cells were fixed with 4 % formaldehyde and stained with 0.1 % crystal violet for 30 min. Next, the cells were rinsed with $dH_2O$ and photographed under a light microscope. The crystal violet stained colonies were dissolved with acetic acid and measured with a microplate reader at 550 nm. The percentage of colony formation in the treatment and control groups was then compared.

**Statistical analysis**

All experimental results were obtained from at least three independent experiments. The data were presented as an average ± a standard deviation (mean ± SD). The Statistical differences between groups were analyzed with One-way ANOVA and Tukey-Kramer Honest for post hoc test using GraphPad Prism software (GraphPad Software; California, USA). A *p*-value of less than 0.05 was considered statistically significant.

## Results and discussion

**Induction of 3T3-L1 preadipocytes into mature adipocytes**

To prove the success of differentiation of 3T3-L1 cells under the stimulation by adipogenic cocktail, the morphological changes and Oil Red O staining were used. Under a light microscope, as seen in **Figure 1**, 3T3-L1 fibroblast-like cells (preadipocyte) could differentiate into mature fat cells on day 10 by expressing large fat droplets within the cells (**Figures 1(A)** and **1(B)**). The Oil Red O staining revealed the mature adipocyte cells in red, which confirmed the presence of lipid droplets (**Figures 1(C)** and **1(D)**). These results indicate that the stimulants successfully differentiated 3T3-L1 fibroblast-like cells into mature adipocytes which were ready to use for further experiments.

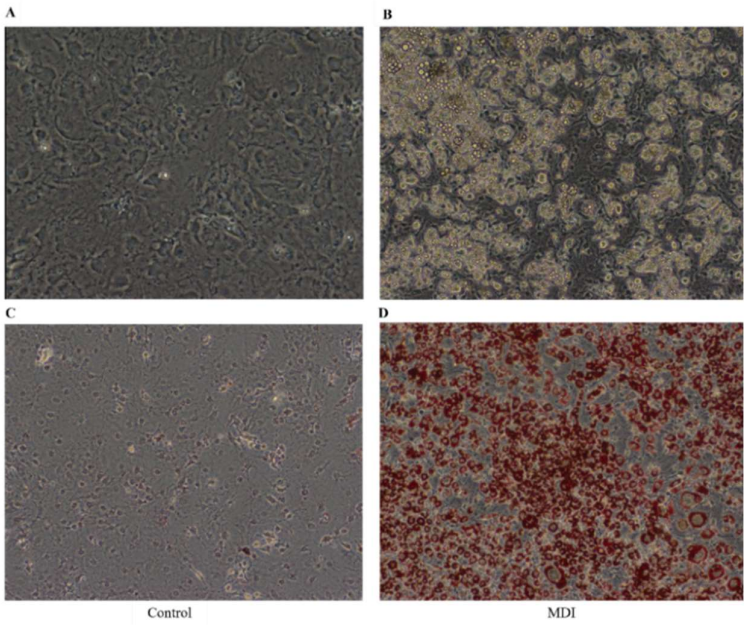


**Figure 1** Morphological characteristics of 3T3-L1cell preadipocytes versus differentiated 3T3-L1 adipocytes ((A), (B)) and after stained with Oil Red O ((B), (C)). The cell images were photographed under a microscope with a 10× objective lens. Cells without stimulation were represented as control, while MDI represented the condition when stimulated by an adipogenic cocktail.

**Cytotoxicity of pinostrobin on 3T3-L1 cells**

To explore the cytotoxicity and determine the suitable concentration of pinostrobin used in this study, 3T3-L1 cells at a concentration of $1\times10^4$ per well were cultured for 24 h until fully grown in a 96-well plate. Next, the cells were then treated with pinostrobin (0, 5, 10, 25 and 50 µM) for 48 h, and the percentage of cell survival was measured by determining the percentage of surviving cells after staining with crystal violet dye (the stained cells indicated living cells). The results revealed that giving pinostrobin at a concentration above 50 µM for 48 h significantly reduced the percentage of cell survival to 78.36 ± 8.14, while concentrations ranging from 0-25 µM were not toxic to the cells with the percentage of cell survival ranging from 100, 102.88 ± 9.39, 99.89 ± 2.34 and 89.33 ± 8.45, respectively (**Figure 2(A)**). Additionally, the cytotoxicity of pinostrobin on 3T3-L1 cells was tested with MTT assays for 48 and 72 h. The results showed that 0 - 50 µM of pinostrobin was not toxic to the cells after 48 h of exposure; however, the cell viability was dramatically reduced in the 25 and 50 µM groups at 72 h (**Figures 2(B)** and **2(C)**). Besides, the cytotoxicity screening of MCF-7 after treatment with pinostrobin at various concentrations ranging from 0 to 100 µM revealed no toxicity to the MCF-7 cells (data not shown) which is in line with the previously published by Wiyono *et al.* [29] indicated that the percent inhibition rate of pinostrobin isolates concentrations of 11.56, 23.12, 46.24, 92.49, 184 and 369.98 µM on MCF-7 were 0.743 ± 5.643, 14.047 ± 2.065, 33.593 ± 6.694, 47.269 ± 1.286, 53.475 ± 4.551 and 57.756 ± 4.814, respectively. The IC50 is approximately 162 µM which is higher than the concentration of pinostrobin affecting 3T3-L1 (~50 µM).

Therefore, the non-toxic concentration of pinostrobin which is below 50 μM treatment for 48 h was selected as an appropriate condition for further studies.

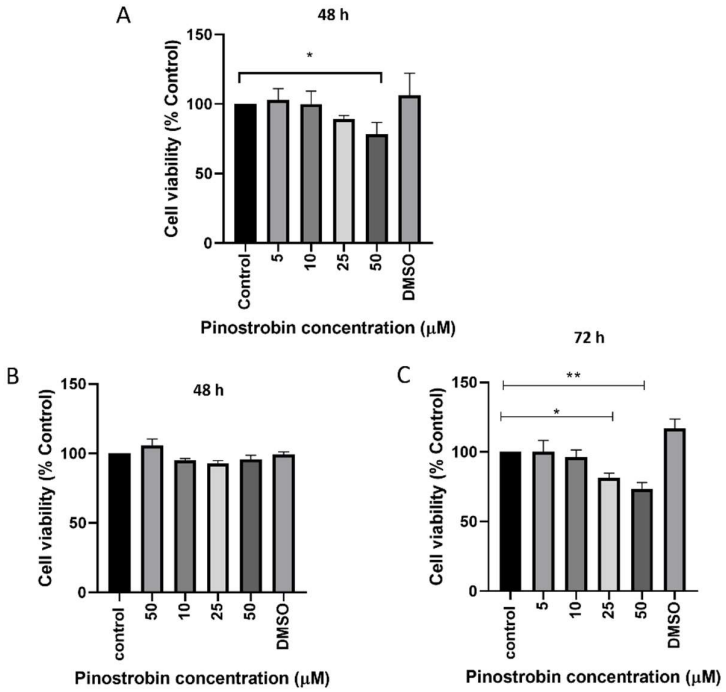


**Figure 2** The percentages of cell viability by crystal violet staining on 3T3-L1 cells treated with pinostrobin at concentrations of 0, 5, 10, 25 and 50 μM for 48 h were presented as mean ± SD (n = 3) (A). The percentage of cell viability by MTT assay treated with pinostrobin at various concentrations for 48 and 72 h was presented as mean ± SD from three independent experiments (B) - (C). The *, **, and *** indicated *p*-values of less than 0.05, 0.01 and 0.001, respectively.

**Pinostrobin inhibits 3T3-L1 adipocyte differentiation**

The findings of several previous studies indicate that the differentiation and migration of preadipocytes are important for adipose tissue development [3,11]. Also, the accumulation and dysfunction of adipocytes surrounding the breast cancer cells play an essential role in promoting cellular proliferation and metastasis [14-16,30-36]. Since the high amount of adipose tissue is related to the progression of breast cancer cells, the reduction of fat cell number by the inhibition of adipogenesis might consequently inhibit cancer cells migration and proliferation. To test the ability of pinostrobin to inhibit adipogenesis, four experimental groups were assigned: Non-differentiated (Control/ND), differentiated with adipogenic cocktail (MDI), MDI + Pinostrobin 10 μM, and MDI + Pinostrobin 25 μM. After 10 days of differentiation, the results showed that the induction of 3T3-L1 cells with an adipogenic cocktail significantly increased the accumulation of intracellular lipids compared with non-differentiated cells. Interestingly, treatment with pinostrobin with concentrations as low as 10 μM tended to reduce the differentiation of preadipocytes into

mature adipocytes, while treatment with 25 µM pinostrobin significantly inhibited adipogenesis. The quantitative results showed that lipid accumulation in the 25 µM pinostrobin treatment group was reduced to 58.16 % (**Figures 3(A)** to **3(C)**). Not surprisingly, treatment with 25 µM pinostrobin showed more consistent results with Oil red O staining in the treatment group by expressing lower fluorescence intensity compared to untreated conditions after staining with Nile red fluorescence (**Figures 4(A) and 4(B)**). These results imply that pinostrobin could inhibit the adipogenesis of 3T3-L1 which is in line with the experiment of San *et al*. [26], indicating that pinostrobin could reduce the process of fat cell production through the reduction of PPARgamma and CEBPalpha, which are critical adipogenic regulators.

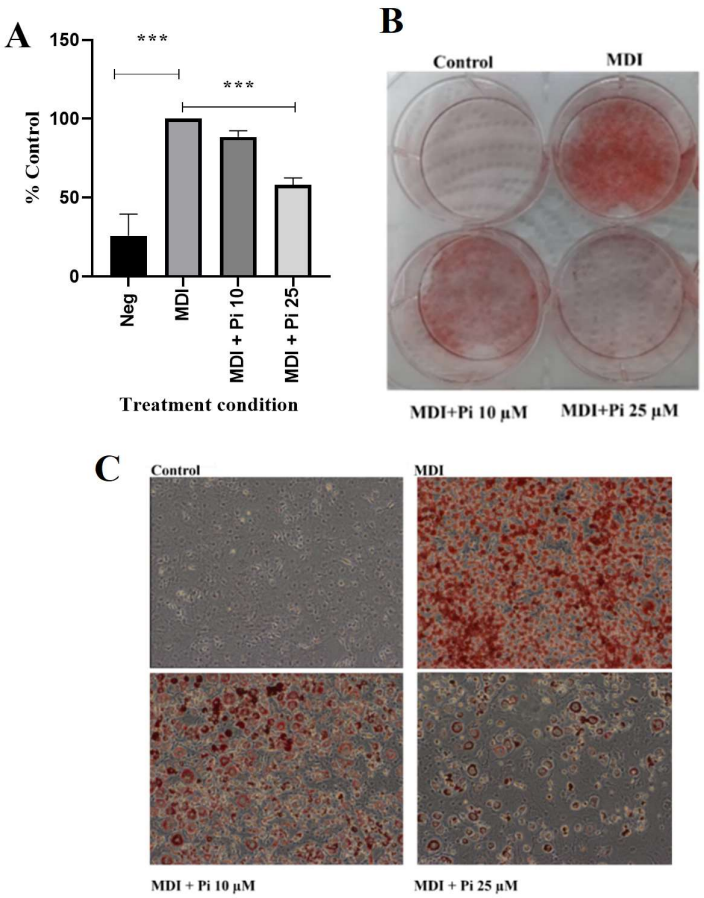


**Figure 3** Pinostrobin inhibits adipocyte differentiation. Oil Red O staining compared between control and treatment conditions (A) - (C). All data are expressed as mean and standard deviation (mean ± SD) (n = 3). A *p*-value of less than 0.05, 0.01, and 0.001, were indicated with *, **, and ***, respectively.

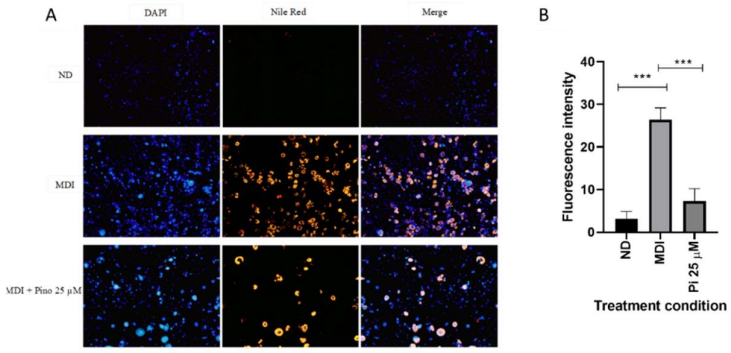


**Figure 4** Pinostrobin inhibits adipocyte differentiation. Nile red and DAPI staining compared between control and treatment conditions were observed under a fluorescence microscope using a 10× objective lens (A). The fluorescence intensities obtained from five independent fields from each condition were calculated using Image J software (B). A *p*-value of less than 0.05, 0.01 and 0.001, were indicated with *, **, and ***, respectively.

**Pinostrobin inhibits 3T3-L1 migration**

The migration of preadipocytes is important in the process of adipose tissue development, particularly in the early step of adipogenesis in which preadipocytes from their original location migrate into cell clusters [4,5]. Several pieces of evidence indicate that excessive migration is correlated with impaired adipose tissue in obese individuals and related to other fibrotic diseases [7-10]. Since we observed that pinostrobin could suppress 3T3-L1 differentiation, the next step is to test whether pinostrobin suppresses the migration of 3T3-L1. To answer this question, 3T3-L1 cells were seeded until 100 % confluency was achieved and treated with 25 μM pinostobin for 6 h. The width of the scratches in each group at 6 h was captured under a light microscope and compared with the width in each group at 0 h. The percentage of the area closed was then calculated and represented as a percentage of control. The results showed that treatment with 25 μM pinostobin could significantly inhibit the mobility of 3T3-L1 cells (**Figures 5(A)** and **5(B)**). Taken together, the antiadipogenic and antimigratory activities of pinostrobin may reduce the accumulation of intra-tumoral adipocytes, which could further reduce the interaction of adipocytes and cancer cells and inhibit adipocyte-induced cancer progression.

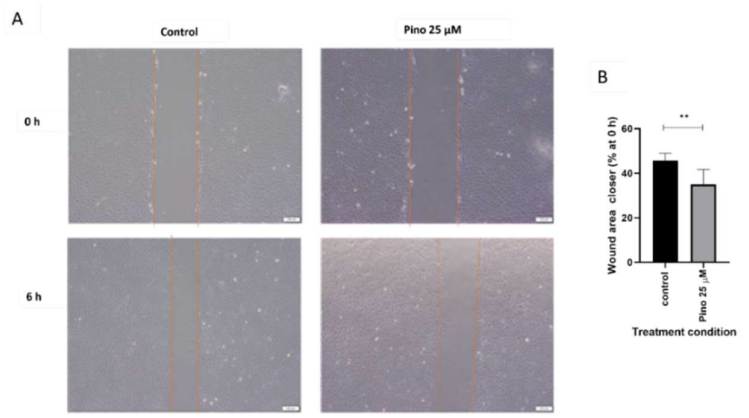


**Figure 5** Pinostrobin reduced 3T3-L1 cell migration at 6 h (3N). The representative pictures of migration assay and the percentage of remaining wound area were compared from three experimental conditions at 0 and 6 h (A) - (B). A *p*-value of less than 0.05, 0.01 and 0.001, were indicated with *, **, and ***, respectively.

**Pinostrobin inhibits adipocyte-induced MCF-7 cell proliferation and migration**

Breast cancer in women is a global health issue that is increasing annually in terms of the number of cases and the mortality rate. Obesity is one of the risk factors for several cancers [37]. The findings of previous research demonstrate that obesity and breast cancer progression are closely correlated with each other and this correlation is known to relate to cancer progression and poor prognosis [3]. Numerous studies have shown that the tumor microenvironment is implicated in tumorigenesis, and it influences the proliferation, invasion, and metastasis of cancer. Among several cell types, adipocytes are known as one of the active components that interact with breast cancer and facilitate the development and progression of the disease via numerous mechanisms, including the secretion of proinflammatory mediators resulting in the promotion of inflammation, hypoxia, and angiogenesis [10,11,14,30,32,33]. To test the inhibitory effects of pinostrobin on the induction of colony formation, 4 experimental groups were assigned: MCF-7 in complete medium, MCF-7 in 70 % CM, MCF-7 in 70 % CM + 10 µM pinostrobin, and MCF-7 in 70 % CM + 25 µM pinostrobin. The results show that MCF-7 in the 70 % CM group had the highest colony formation rate compared to the four groups. Interestingly, treatment with pinostrobin could significantly reduce the growth and the formation of MCF-7 colonies at a concentration as low as 10 µM, with the number of colonies formed being inversely correlated with the concentration of pinostrobin (**Figures 6(A)** to **6(C)**).

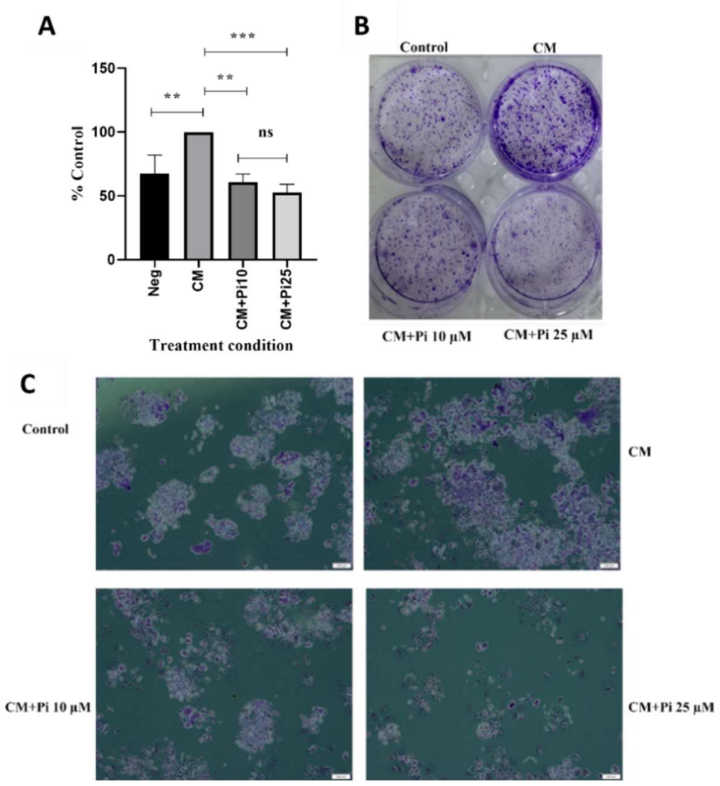


**Figure 6** Pinostrobin reduced breast cancer cell growth and colony formation (3N). The representative pictures of colony formation under four experimental conditions at 16 days were compared (A) - (C). A *p*-value of less than 0.05, 0.01 and 0.001, were indicated with *, **, and ***, respectively.

To explore whether pinostrobin could inhibit the migration of adipocyte-induced MCF-7 breast cancer cells, the indirect co-culture system was used and MCF-7 cells were divided into three experimental groups: MCF-7 in complete medium, MCF-7 in 70 % adipocyte condition medium (CM), and MCF-7 in 70 % adipocyte condition medium (CM) + 25 µM pinostrobin. When comparing the migration of the cells at 0 and 6 h after treatment, the MCF-7 cultured in 70 % CM had the highest rate of cell mobility, caused by a reduction in the area of each scratch from 100 to 72.57 % ($p < 0.05$), while in the pinostrobin treated group, it was reduced to 80.67 ($p < 0.05$) (**Figures 7(A)** and **7(B)**).

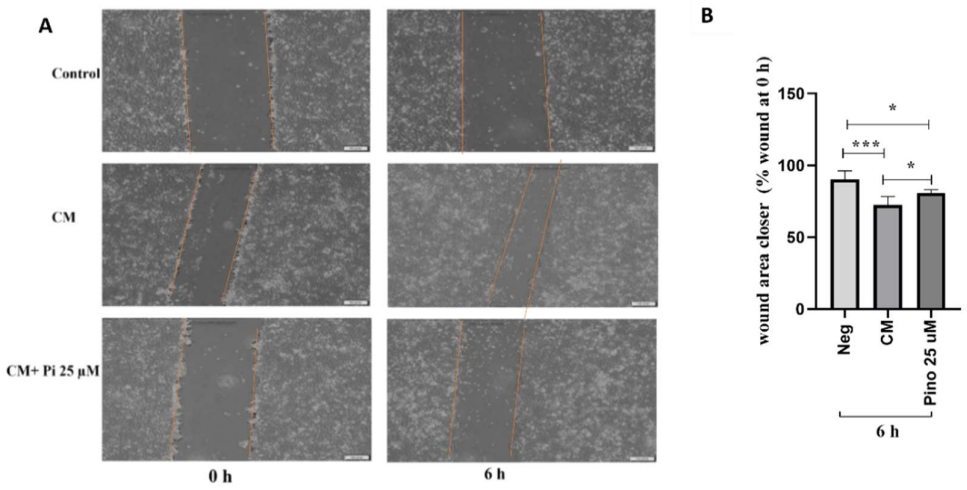


**Figure 7** Pinostrobin reduced breast cancer cell migration at 6 h. The representative pictures of migration assay and the percent of wound area closure were compared from three experimental conditions at 0 and 6 h (A) - (B). All data were expressed as mean and standard deviation (mean ± SD). A *p*-value of less than 0.05, 0.01 and 0.001, were indicated with *, **, and ***, respectively.

In this study, we used an indirect co-culture of 3T3-L1 and MCF-7 to investigate the effects of pinostrobin on adipocytes-induced breast cancer progression. Our results found that MCF-7 cultured in an adipocytes-conditioned medium promoted MCF-7 proliferation and migration (**Figures 6** and **7**) which is consistent with the findings from Gyamfi et al. [38], who found that using a co-culture of adipocytes with cancer cells promotes cancer cell invasion and migration through the induction of epithelial-mesenchymal transition (EMT). In addition, the study of Manabe *et al.* [31] also concurred with our results, demonstrating that an indirect co-culture of mature adipocytes and the MCF-7 - an ER+ breast cancer cell - promoted the growth of these cancer cells [14].

Recently, the advancement of breast cancer therapeutics has improved significantly. Nevertheless, myriad limitations still exist [13]. Therefore, new treatment approaches are still in need. The therapeutic compounds extracted and derived from commonly used plants or herbs have been reported to inhibit many types of cancer cells, both *in vitro* and in animal models, through several mechanisms and these dietary interventions in managing cancer treatments have gained tremendous attention [20-23,38-42]. In our study, we used pinostrobin extracted from *Boesenbergia rotunda* (*L.*). Pinostrobin has been known to exert several pharmacological effects, including anti-cancer properties [24-26,29]. However, the effects of pinostrobin on inhibiting the proliferation and migration of breast cancer cells in adipocyte-induced conditions are still unknown. We are the first to report that pinostrobin, a compound extracted from *Boesenbergia rotunda* (*L.*) at a concentration of 25 µM, significantly inhibits the proliferation and migration of breast cancer cells in adipocyte-induced conditions in terms of reducing both the mobility and colony formation of MCF-7 breast cancer cells. It was further noted that a low concentration of pinostrobin (i.e., 10 µM) could significantly

inhibit the formation of MCF-7 colonies. Therefore, it can be concluded from the results of this study that pinostrobin extracted from *Boesenbergia rotunda* (*L.*) has promising anti-adipocyte-induced MCF-7 progression effects (**Figures 5** and **6**). However, more studies should be conducted to explain how pinostobin affects breast cancer cells across different cancer types and stages. Additionally, the precise molecular mechanisms underlying the effects of pinostrobin in the context of the co-culture system need to be further explored. For example, the investigation of the expression of migratory-related markers, apoptosis-related markers, and the signaling cascades that play a role in cancer aggravation such as TGFβ Signaling, WNTβ Catenin Signaling, and Notch signaling [14]. Another critical mechanism related to adipocytes and cancer cell progression is the ability of the surrounding adipocytes to fuel cancers through the transmission of free fatty acids (FFA) via carrier proteins [11]. The FFAs taken up by the cancer cells can either act as substrates for metabolic pathways or be stored in small LDs and elevate reactive oxygen species (ROS), which then further induce tumor cell invasion through the hypoxia-inducible factor 1/MMP14 signaling pathway [11]. These molecular pathways may account for the inhibitory effects of pinostrobin in the context of adipocyte-induced breast cancer progression and the effects of pinostrobin on these mechanisms need to be explored before any conclusion can be drawn. Apart from an *in vitro* study, the investigations elucidate pinostrobin's efficacy *in vivo* using animal models to assess its safety profile and efficacy in more complex biological systems as well as finding the potential synergistic effects with existing treatments that need to be done in the future.

## Conclusions

This study has shown that pinostrobin could inhibit adipocyte-induced proliferation and migration of ER+ breast cancer cells in the context of a co-culture system with 3T3-L1 cells. In addition, pinostrobin could also inhibit adipogenesis. However, further studies need to be conducted to investigate the underlying mechanism of the inhibition and its possibility as a novel therapeutic agent for breast cancer treatment in obesity-associated conditions.

## Acknowledgments

We would like to thank Associate Professor Dr. Darawan Pinthong, Department of Pharmacology, Faculty of Science, Mahidol University, Thailand, for providing the MCF-7 cell line. We also thank all members of the Research Center of Transporter Protein for Medical Innovation, Department of Physiology, Faculty of Science, Mahidol University, for kindly providing the chemicals and equipment used in this study. The research funding supporting this study was provided by the Faculty of Medicine, Princess of Naradhiwas University to Ameena Benchamana.

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