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ORIGINAL ARTICLE

Effect of *Piper nigrum* Ethanolic Extract on Human Breast Cancer Cell Growth and Cell Migration

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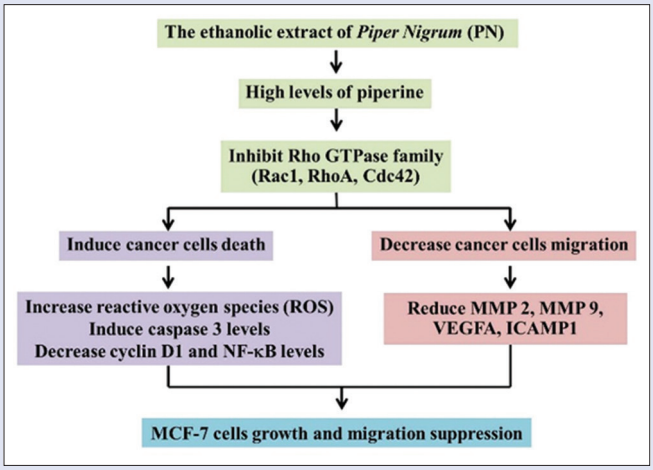
ABSTRACT

Background: *Piper nigrum* (PN) is widely used as a traditional medicine, which has anti-cancer activity among others. **Objective:** Our study purposes to illuminate the inhibition of PN effects on breast cancer cells growth and migration along with its mechanisms. **Materials and Methods:** The piperine in the young fruit of PN extract was determined by the high-performance liquid chromatography method. Growth inhibition with a mechanism of PN extract was studied in the MCF-7 cells using sulforhodamine B assay, cell cycle analysis, colony formation, caspase-3 activity, and reactive oxygen species formation. Furthermore, the anti-migratory effects of PN were investigated using wound healing, matrigel migration, and gelatin zymography assay. Finally, PN mechanism was determined by reverse transcription polymerase chain reaction and Western blotting for the gene and protein levels. **Results:** Piperine level was showed at a high concentration in the PN extract. Further, the PN extract suppressed Rac1 mRNA expression in the mevalonate (MVA) pathway as well as repressed Rac1 and RhoA protein expression. Interestingly, PN stimulated growth inhibition in dose- and time-dependent as well as being accompanied by increasing the G1 phase arrest and inhibiting cyclin D1 and NF-κB as well as inducing caspase-3 expression. The PN extract inhibited MCF-7 cell migration by reducing matrix metalloproteinases (MMP) 9 protein expression as well as MMP 2, MMP 9, VEGFA, and ICAMP1 gene expression. **Conclusion:** PN could be herbal medicine for anti-cancer and anti-migratory activities with a correlation to the MVA pathway; therefore, PN maybe deserving further to study as a new candidate for treating breast cancer.

Key words: Cell migration, MCF-7 cells, mevalonate pathway, *Piper nigrum*, piperine

SUMMARY

- PN extract found potential anti-cancer and anti-migratory activity against MCF-7 cells human breast cancer.



Abbreviations used: AI: *Azadirachta indica*; CS: *Careya sphaerica*; CA: *Centella asiatica;* CT: *Clitoria ternatea*; DHE: Dihydroethidium; MMP: Matrix metalloproteinases; MC: *Morinda citrifolia;* MO: *Moringa oleifera*; OI: *Oroxylum indicum*; PN: *Piper nigrum;* PS: *Piper sarmentosum*; ROS: Reactive oxygen species; SRB: Sulforhodamine B; TL: *Thunbergia laurifolia*.

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INTRODUCTION

Piper nigrum (PN) belongs to family Piperaceae and is commonly known as black pepper. It is broadly used in folk medicine in many countries in the south of Asia, and it is famously well-known as the king of spices.[1] PN belongs to family Piperaceae and is commonly known as black pepper. It has many active compounds including phenolics, flavonoids, terpenes, lignans, steroids, and alkaloids as well as piperine, pipernonaline, and purpurogallin.[2] PN is reported to inhibit bacterial, fungal, diarrheal, inflammatory, metastatic, and cancer activities.[2] For anti-cancer effects, PN inhibited cell growth in many cancer cell types, such as colon,[3] lung,[4] and breast[5,6] through increasing G2/M phase arrest along with a reduction in the levels of expression of cyclin B1 and induction of cyclin-dependent kinase-1.[7] Moreover, the active compounds of PN, such as pipernonaline, showed apoptotic effects through causing reactive oxygen species (ROS) production and activating caspase-3 activity in prostate cancer cells.[8] Piperine suppressed the matrix metalloproteinases (MMP) including MMP 2 and MMP 9 activity and further piperine also induced the tissue inhibitors of metalloproteinase 1/2 levels.[7] However, the effects of PN on the mevalonate (MVA) pathway are still unclear.

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The MVA pathway correlates with cancer cell proliferation and migration through using cholesterol products to grow[9] or consuming high cholesterol levels to maintain their metabolism.[10] Inhibitors of the MVA pathway caused cell proliferation inhibition by reducing isoprenoid precursors, farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP).[9] FPP and GGPP are essential for posttranslational modification of the small G-proteins, including Rac, Rho, and Ras, which regulate the mechanisms in many cancer cells, such as cellular transformation, cell differentiation, cell proliferation, cell migration, and cell invasion.[11] At present, MVA synthesis is reduced by statin drugs through the inhibition of the HMG-CoA reductase activity and then leading to the inhibition of cancer cell growth and migration.[12] Nevertheless, less information is identified about the effects of herbal medicine or PN on the MVA pathway in breast cancer cells. Therefore, PN effects are very interesting to explore on the MVA pathway.

Accordingly, several studies are an effort to screen the edible natural products or plants to determine the new anti-cancer effects. In the present work, we focused on the young fruit of PN extract in MCF-7 breast cancer cells inhibition with the precise mechanism(s) responsible, including MVA pathway. In conclusion, our work could be helpful in the discovery of the new plants on MVP pathway that more efficacy and lower toxicity regimens for breast cancer therapy.

MATERIALS AND METHODS

Herbal preparation and extraction

Young fruit of PN L. was obtained from Maha Sarakham Province, Thailand, in April 2017. Proof of identity was made by the Department of Applied Thai Traditional Medicine, Faculty of Medicine, Mahasarakham University (specimen no. MSUT_7234) and placed at the Faculty of Science, Mahasarakham University, Thailand. To prepare/ethanolic extract, young fruit PN fruits were weighed (250 g), dried and then soaked in 95% ethanol for 7 days. Then, extracts were filtered, evaporated, and lyophilized by lyophilizer under the pressure. Percentage yield of the extracts are 5.27% per dry weight of PN young fruits.

Cell cultures and cell death method

The human MCF-7 breast cancer cell lines were purchased from the American Type Culture Collection (ATCC; USA) and then continued culture in accordance with ATCC's recommendations. PN extracts on the cancer cells viability were performed by sulforhodamine B (SRB) method as in a previously described method.[13,14] MCF-7 cell was exposed to the PN extract about 0–100 μg/mL for 24–48 h. Consequently, the cell was then stained with 0.4% SRB dye, added with 10 mm Tris base buffer to solubilize, and the optical intensitied were measured at 540 nm.

Colony formation assay

PN extracts on the cancer cells replication were performed by colony formation method as in a previously described method.[13,14] MCF-7 cell was exposed to the extracts about 0–100 μg/mL for 24 h added to the new medium and grown up for 2 weeks to form the colonies. Cells were fixed, stained, and colonies were viewed and counted.

Reactive oxygen species formation

PN extracts on the ROS formation was performed using cell-permeable fluorescent probe, dihydroethidium (DHE).[13,14] MCF-7 cell was exposed to the extracts about 0–500 μg/mL with 25 μM DHE in serum-free medium and kept in a 5% CO_2 atmosphere at 37°C for 90 min in the dark. Fluorescence intensity was measured at 518 nm (excitation) and 605 nm (emission) on a fluorescent microplate reader. The data were expressed as the percentage of ROS relative to untreated controls.

Caspase-3 activity

PN extracts on the caspase-3 activity were performed using fluorimetric assay kits (Sigma-Aldrich; Merck KGaA) according to the manufacturer's protocol.[13,14] MCF-7 cell was exposed to the extracts about 0–100 μg/mL for 24 h, and cells were lysed with RIPA lysis buffer. The caspase-3 mixture composed of 5 μL cell lysates (0.5 mg/ml), 195 μL of buffer containing an Ac-DEVD-7-amino-4-methylcoumarin (AMC) – conjugated substrate and incubated for 90 min incubation at 37°C in the dark. The fluorescence intensity was read at excitation and emission wavelengths of 360 and 460 nm, respectively and using AMC standard to calculate caspase-3 activity.

Wound healing method

PN extracts on the cell migration were measured by a wound healing method as described previously.[13,14] MCF-7 cells were made a wound using 200 μL pipette tip and then added to the new medium containing the PN extract (0–5 μg/mL) for 48 h. After that, images of the wounds were captured for comparison between 0 and 48 h.

Matrigel migration method

PN extract on migration was done by matrigel migration method in a 24-well Transwell chamber (8 μM pore size; Corning, Lowell, MA). MCF-7 cells were seeded about 2.5×10^4 cells/well into the Transwell chamber with the PN extract about 0–100 μg/mL in DMEM medium containing 0% fetal bovine serum, the lower part was added with complete medium and cultured for 24 h. Afterward, the transwell was removed, fixed with 100% methanol for 30 min, stained with 0.5% crystal violet, washed several times with tap water and then photographed with inverted microscopy (×10 magnification).

Gelatin zymography method

PN extract on migration was measured by gelatin zymography analysis as previously described.[13,14] MCF-7 cells were treated with PN extract about 0–100 μg/mL for 48 h, the medium was collected, and the protein concentrations were examined. Proteins were combined with ×2-non-reducing buffer and loaded onto 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with gelatin (0.01% for final concentration). The gel was exposed to 2.5% Triton X-100 for 30 min, developing buffer for overnight, 0.5% Coomassie Brilliant Blue R-250 for 1–2 h, and washed with a destaining buffer for 24 h.

Cell cycle analysis

PN extracts on the cancer cells progression were determined by cell cycle analysis as previously explained.[15] MCF-7 cell was exposed to the various concentrations of PN extracts (0–25 μg/mL) for 24 h, harvested and fixed with 70% cold ethanol for 24 h. Afterward, cells were washed with phosphate buffered saline buffer and then mixed with 0.02 mg/mL PI for 1 h at 4°C. The content of DNA was investigated by a flow cytometer (Guava Technologies, Hayward, CA) and the percentages of cell cycle phases were measured using the Guava EasyCyte system (Guava Technologies, Hayward, CA, USA).

Reverse transcription polymerase chain reaction method

PN extracts on the targeted gene expression were determined by reverse transcription polymerase chain reaction (PCR) as explained previously.[13,14] MCF-7 cells were exposed to the PN extract about 0–50 μg/mL for 24 h, mRNA was extracted using Trizol® reagent and then reversed to complementary deoxyribonucleic acid. The PCR was investigated using target primers, as shown in Table 1. The differences of gene expression were estimated using the $2^{-\Delta\Delta Cq}$ method for calculating relative quantification, and the expression levels of the target gene were compared with control groups.

Western blotting method

PN extracts on the targeted protein expression were performed by Western blotting as previously described.[13,14] Cells were exposed to the PN extract about 0–50 μg/mL for 24 h, the proteins were extracted using RIPA lysis buffer, centrifuged, and collected the supernatant protein. Protein was subject to SDS-PAGE, transferred to a Polyvinylidene difluoride membrane (Immobilon®, Millipore, USA). The membranes were blocked with 2.5% (w/v) bovine serum albumin and exposed overnight with the primary antibodies (Rac1, RhoA, cyclin D1, caspase-3, NF-κB, and beta-actin). The next day, the membrane was washed, exposed to a secondary antibody at room temperature for 2 h, and the target protein was detected by Clarity™ Western ECL Substrate. The protein densities were detected and took pictures by ChemiDoc™ Touch Imaging System (Bio-Rad).

Table 1: Primer sequences for reverse transcription polymerase chain reaction

Gene symbol	Primer sequences
Rac1	Forward primer 5' ATG-TCC-GTG-CAA-AGT-GGT-ATC 3'
	Reverse primer 5' CTC-GGA-TCG-CTT-CGT-CAA-ACA 3'
RhoA	Forward primer 5' GGA-AAG-CAG-GTA-GAG-TTG-GCT 3'
	Reverse primer 5'GGC-TGT-CGA-TGG-AAA-AAC-ACA-T 3'
Cdc42	Forward primer 5'CCA-TCG-GAA-TAT-GTA-CCG-ACT-G 3'
	Reverse primer 5' CTC-AGC-GGT-CGT-AAT-CTG-TCA 3'
MMP 2	Forward primer 5' GAT-ACC-CCT-TTG-ACG-GTA-AGG-A 3'
	Reverse primer 5' CCT-TCT-CCC-AAG-GTC-CAT-AGC 3'
MMP 9	Forward primer 5' GGG-ACG-CAG-ACA-TCG-TCA-TC 3'
	Reverse primer 5' TCG-TCA-TCG-TCG-AAA-TGG-GC 3'
ICAM1	Forward primer 5' GTA-TGA-ACT-GAG-CAA-TGT-GCA-AG 3'
	Reverse primer 5' GTT-CCA-CCC-GTT-CTG-GAG-TC 3'
VCAMA	Forward primer 5' AGG-GCA-GAA-TCA-TCA-CGA-AGT 3'
	Reverse primer 5' AGG-GTC-TCG-ATT-GGA-TGG-CA 3'
Beta-actin	Forward primer 5' GTG-ACG-TTG-ACA-TCC-GTA-AAG-A 3'
	Reverse primer 5' GCC-GGA-CTC-ATC-GTA-CTC-C 3'

MMP: Matrix metalloproteinases

High-performance liquid chromatography method

A high-performance liquid chromatography (HPLC) assay was used to measure the piperine of the PN extracts as described previously with minor modifications.[16] The PN extract was loaded onto Inertsil ODS-3 C_{18} analytical column (GL Sciences Inc., Tokyo, Japan). Mobile phase composed of acetonitrile: DW: Acetic acid (60.0:39.5:0.5), the injection was performed using a 20 μL loop at 340 nm.

Statistical analysis

The difference between control and treatment groups was statistically compared by the Student's *t*-test and $P < 0.05$.

RESULTS

Piperine in *piper nigrum* extract

Piperine is the most abundant alkaloid in PN, and it is the active anti-cancer compound. To examine the piperine content in the PN extract, we used the HPLC method. The data revealed the presence of piperine in the PN extract, as shown in Figure 1, to be 107.09 ± 15.24 μg/mL of piperine/dry weight extract.

Piper nigrum extract on Rac1, RhoA, and Cdc42 expression

Rac1, RhoA, and Cdc42 are the Rho GTPase family that is involved in regulating a variety of normal and cancer cellular processes. Importantly, the Rho GTPase genes are unregulated in many cancer cells and may control the cancer cells' proliferation. The PN extract reduced the Rac1 gene expression significantly at 50 μg/mL. On the other hand, the PN extract slightly decreased the RhoA and Cdc42 genes expressions, but no significance was found at 25–50 μg/mL [Figure 2a].

Further, the PN extract treatment at 10–50 μg/mL generated a dramatic reduce in the Rac1 and RhoA protein expression levels [Figure 2b]

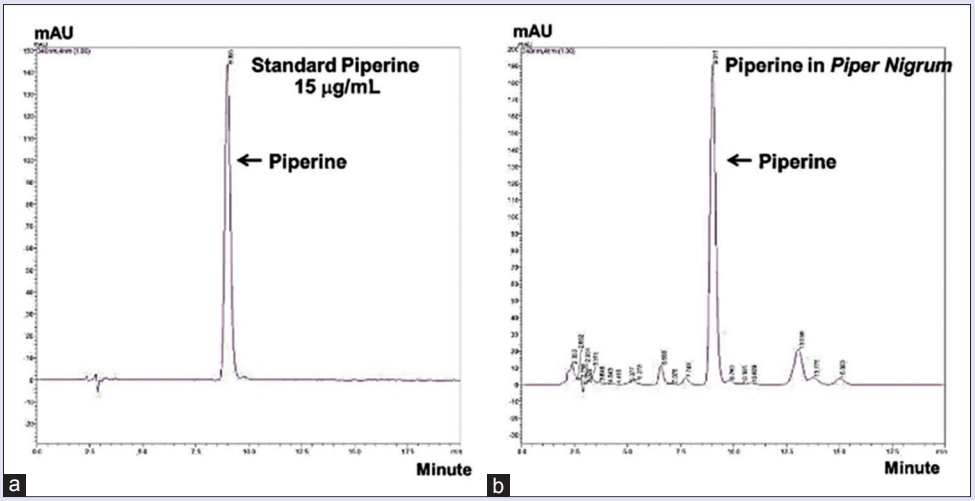


Figure 1: High-performance liquid chromatography chromatogram of piperine in *Piper nigrum* extracts, (a) piperine standard (b) piperine in *Piper nigrum*

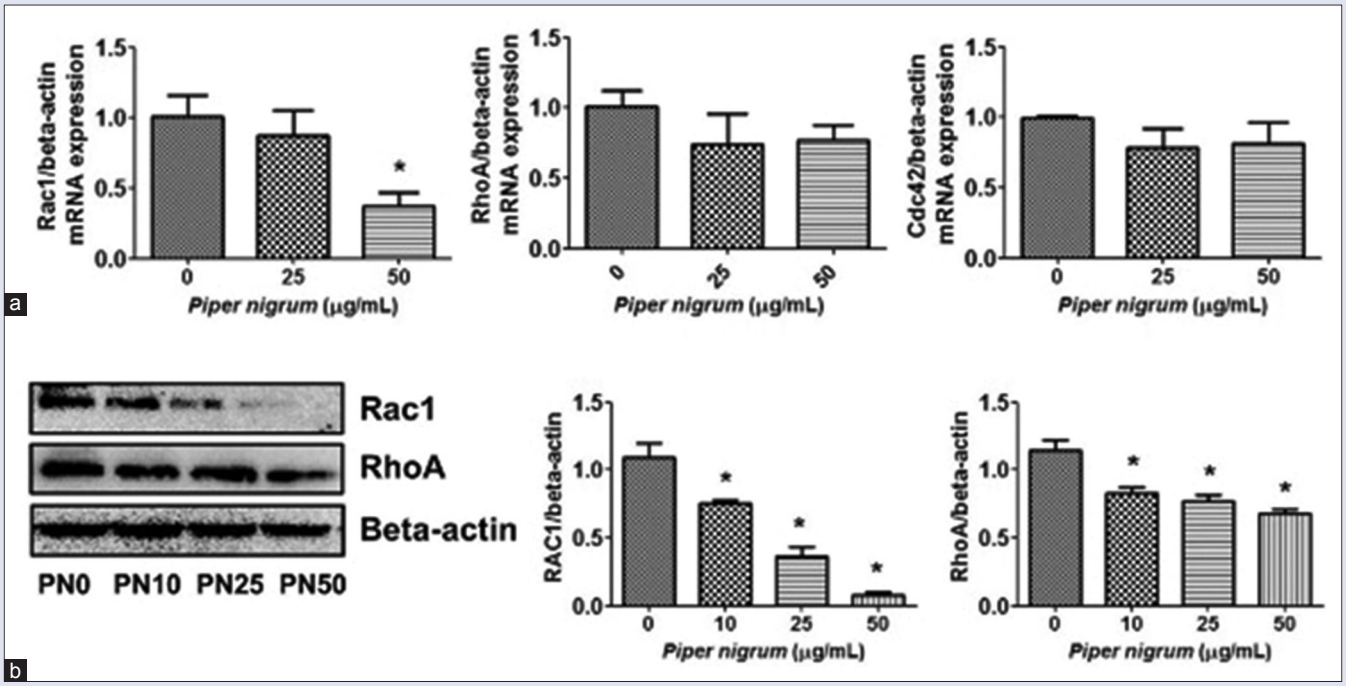


Figure 2: Effects of PN extract on gene and protein expressions of Rac1, RhoA, and Cdc42 (a and b). MCF-7 cells were treated with 25–50 µg/mL PN extract for 24 h and then analyzed gene and protein expression. Reverse transcription polymerase chain reaction and Western blotting were carried out for targeted gene and protein expression, respectively. Data represent mean ± standard error of the mean of three independent experiments. *$P < 0.05$ versus control. PN: *Piper nigrum*

expression significantly at 10–50 µg/mL. In conclusion, the PN extract can interrupt the important step of cholesterol synthesis or the MVP pathway, and this maybe the mechanism of the PN extract to induce the breast cancer cell death.

Piper nigrum extract inhibited MCF-7 cells growth

The results indicated that PN extracts utilized a forceful MCF-7 cells death with IC_{50} values of 42.2 ± 5.8 and 15.6 ± 2.1 µg/mL for 24–48 h [Figure 3a and b]. In addition, PN extract produced an increase G1 phase arrest in a dose-dependent manner with show a significant result at the dose of 10 µg/mL and strongly effect at 25 µg/mL [Figure 3c] and also cells were decreased at S phase. Further, PN extracts triggered the colony forming ability of cancer cells and found the significant at 25–100 µg/mL [Figure 3d and e]. In conclusion, the PN extract can inhibit the MCF-7 cell growth, induce the cancer cell cycle arrest, and inhibit the colony formation. Our data showed that the PN extract induced breast cancer cell death, and the mechanism of action will be explored.

Piper nigrum extract on reactive oxygen species production and caspase-3 activity

Subsequently, MCF-7 cell was exposed to PN extract, we found that ROS formations were augmented in a dose-dependent that were significant at 500 µg/mL [Figure 4a] after 90 min incubation. Consistency with caspase-3 activity, the PN extract showed a significant to increase the caspase-3 activity (25–100 µg/mL) after incubation with the PN extract for 24 h [Figure 4b]. In conclusion, the PN extract activates the ROS formation, increases caspase-3 activity, and induced breast cancer cell death.

Piper nigrum extract on the expression of proteins associated with cell cycle

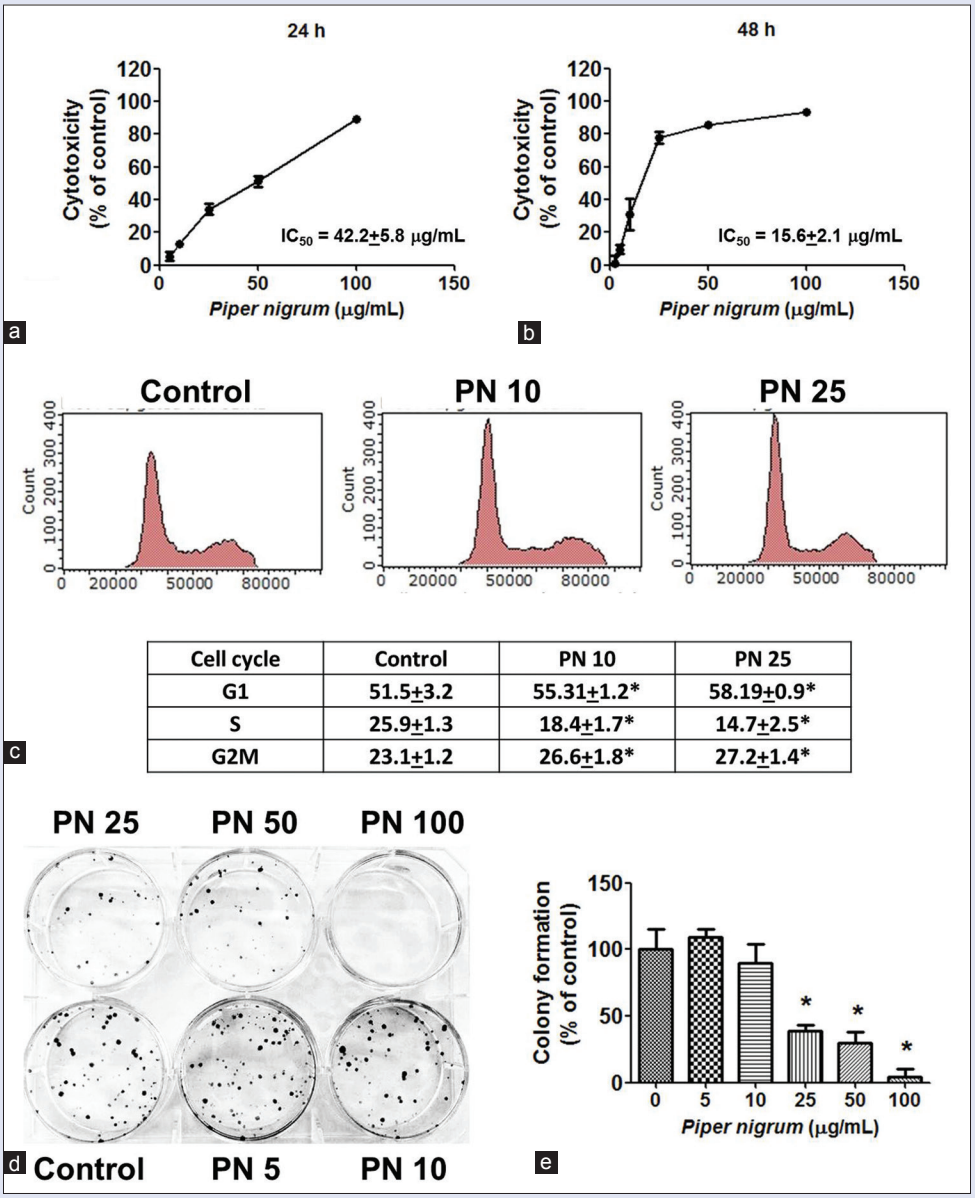
After MCF-7 cells were exposed to the PN extract, we found that PN caused a significant cyclin D1 reduction [10–50 µg/mL; Figure 4c]. On the other hand, caspase-3 protein levels were also significantly augmented [Figure 4c]. Finally, PN decreased the NF-κB, which controls the inflammation, cell proliferation, and cell growth and many protein levels [Figure 4c]. In conclusion, the PN extract caused induction of breast cancer cell death by reducing cyclin D1 and NF-κB and increasing caspase-3 protein expressions.

Piper nigrum extract inhibited breast cancer cells migration

For the wound healing methods, our results indicated that PN inhibits MCF-7 cells migration and showed the significant effects at a dose of 2.5–5 µg/mL PN [Figure 5a]. For the matrigel migration method, the PN extract significantly inhibited MCF-7 cell migration to the bottom chamber [Figure 5b]. For the gelatin zymography method, PN at a dose of 100 µg/mL showed a significant reduction of MMP 9 levels [Figure 5c]. In conclusion, the PN extract caused inhibition of breast cancer MCF-7 cell migration in a dose-dependent manner and showed the significant levels at the lower concentration.

Piper nigrum extract on matrix metalloproteinases 2, matrix metalloproteinases 9, VEGFA, and ICAMP1 expressions

Our results indicated that the PN extract significantly inhibited MMP 2 expression at a dose of 50 µg/mL. Moreover, the PN extract had the greatest ability to suppress MMP 9, VEGFA, and ICAMP1 gene



Cell cycle	Control	PN 10	PN 25
G1	51.5±3.2	55.31±1.2*	58.19±0.9*
S	25.9±1.3	18.4±1.7*	14.7±2.5*
G2M	23.1±1.2	26.6±1.8*	27.2±1.4*

Figure 3: Effects of PN extract on viability, cell cycle progression and colony formation of breast cancer cells (a-e). MCF-7 cells were treated with 0–100 µg/mL PN extract for 24–48 h and then analyzed cancer cells death by sulforhodamine B. Cells were treated with 0–25 µg/mL PN extract for 24 h and then analyzed cell cycle arrest by flow cytometry. Cells were treated with 0–100 µg/mL PN extract for 24 h and then added the new complete medium every 2 days for 2 weeks and analyzed colony formation. Data represent mean ± standard error of the mean of three independent experiments. *$P < 0.05$ versus control. PN: *Piper nigrum*

expressions when compared with MMP 2 expression at the same doses [Figure 6a-d]. In conclusion, the PN extract inhibited MCF-7 cell migration by reducing MMP 2, MMP 9, VEGFA, and ICAMP1 genes expressions.

DISCUSSION

In the present study, the researchers tried to find out the anti-cancer agents from natural resources or plants which are edible plants and low toxicity, such as PN.[17,18] In this study, we explored the PN effects on the breast cancer cells growth and migration and its mechanism. Our results illustrated that a PN extract caused the inhibition of MCF-7 cells growth and migration by the dose-dependent manner. Based on the results obtained, we estimated the active compound of PN, piperine, using the HPLC method, and then, we found that the PN extract had the highest levels of piperine. Next, to explore the PN effects on the MVA pathway and then gene and protein expressions were used. The results showed that the PN extract down-regulated the Rac1 gene expression as well as Rac1 and RhoA protein expression levels. Further, we found that the PN extract can inhibit the proliferation and cell cycle arrest, which were consistent with the reduction of the metastatic potential of MCF-7 cells. In addition, the PN extract also increased the ROS formation and caspase-3 activity along with inhibiting cyclin D1 and NF-κB as well as inducing the caspase-3 protein expression levels. Moreover, the PN extract inhibited migration through reducing the protein expression levels

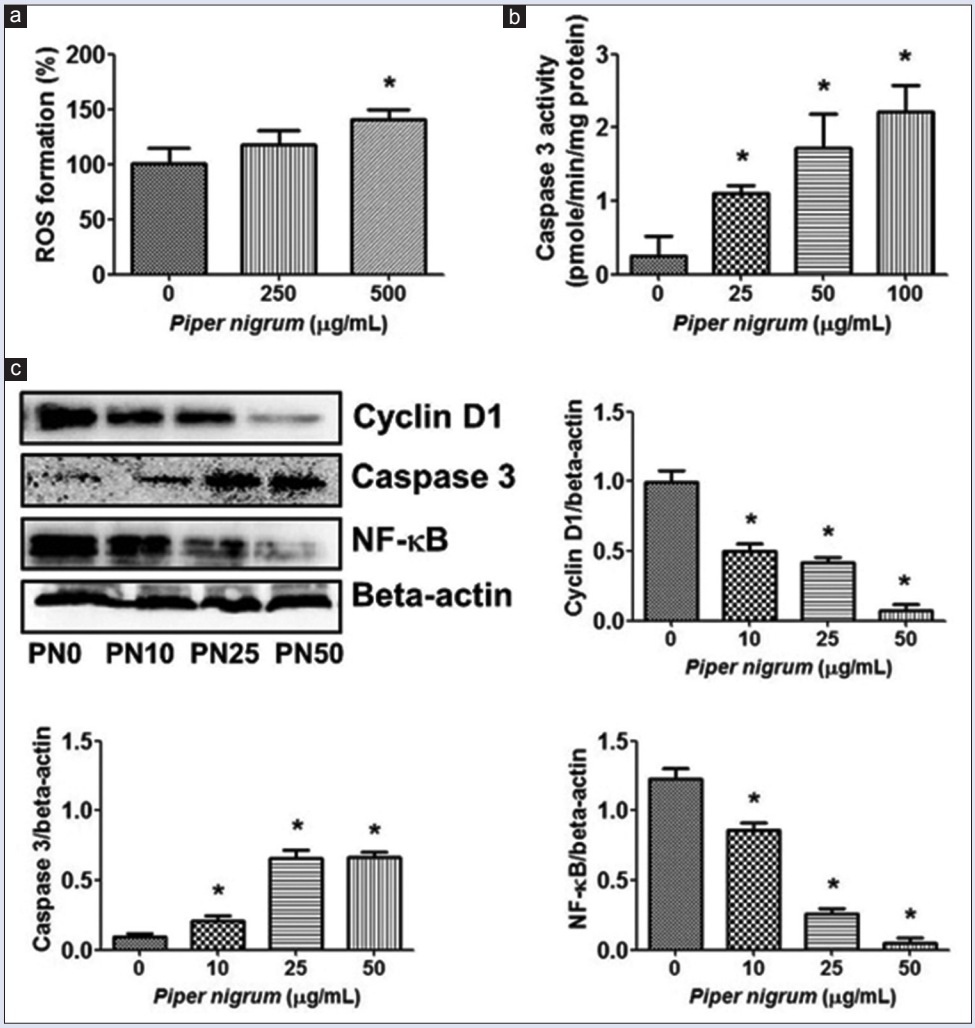


Figure 4: Effects of PN extract on Reactive oxygen species formation, caspase-3 activity and protein-related cell growth or cell death of breast cancer cells (a-c). After MCF-7 cells were treated with 0–500 μg/mL PN extract with DHE-probe for 90 min in dark and analyzed Reactive oxygen species formation by measured fluorescent intensity. Cells were treated with 0–100 μg/mL PN extract for 24 h and measured caspase-3 activity by the kits. Cells were incubated with 0–50 μg/mL PN extract for 24 h and measured protein expression by Western blotting. Data represent mean ± standard error of the mean of three independent experiments. *$P < 0.05$ versus control. PN: *Piper nigrum*

of MMP accompanied with reducing the gene-related migration, MMP 2, MMP 9, VEGFA, and ICAMP1. Therefore, PN may be an attractive herbal medicine for preventing and/or treating for breast cancer.

Essential activities of anti-cancer agents are to against uncontrollable cancer cell growth and migration. A previous study indicated that the anti-proliferative activity of the PN extract and their active components defeat many cancer cells types such as lung, prostate, colon, breast, and osteosarcoma. Usually, the researchers used the ripe PN fruit to study the anti-cancer effects, and the effects showed high efficacy but, in this study, we try to use the young PN fruit as we eat in daily life to use as chemoprevention. For more information, several works indicated that unripe/young PN fruit caused induced many cancer cell types such as colorectal carcinoma cells.[3] In addition, piperine is a major pungent alkaloid and showed antiproliferative effects against many cancer cells.[19] Our study showed that the ethanolic extract of the PN extract had a high concentration of piperine. The anti-cancer effects of PN may be attributed to alkaloids, which are found in PN, for example, pellitorine, purpurogallin, piperlongumine, and pipernonaline.[8,20,21] Additional studies are needed to examine the precise bioactive compounds with anti-breast cancer efficacy in PN. On the other hand, to investigate the MVA pathway to understand the antiproliferative mechanism of the PN extract in breast cancer cells. There is less evidence for the PN extract and MVA pathway. Our results showed that the PN extract reduced the Rac1 gene as well as decreased Rac1 and RhoA protein levels. On the other hand, Lang *et al.* showed that salvicine treatment in human breast cancer cells inhibited RhoC mRNA and protein expression in a dose- and time-dependent manner but had no obvious effect on the levels of RhoA, Rac and cdc42 in MDA-MB-435 cells.[22] In conclusion, blocking the MVA pathway can suppress the growth and migration of breast cancer cells.

Proliferation or regrowth of cancer cells, which are controlled by the cell division/cycle, is the main target of several anti-cancer agents or natural products. Then, we explored the PN effect on MCF-7 breast cancer cell cycle with flow cytometry analysis. The

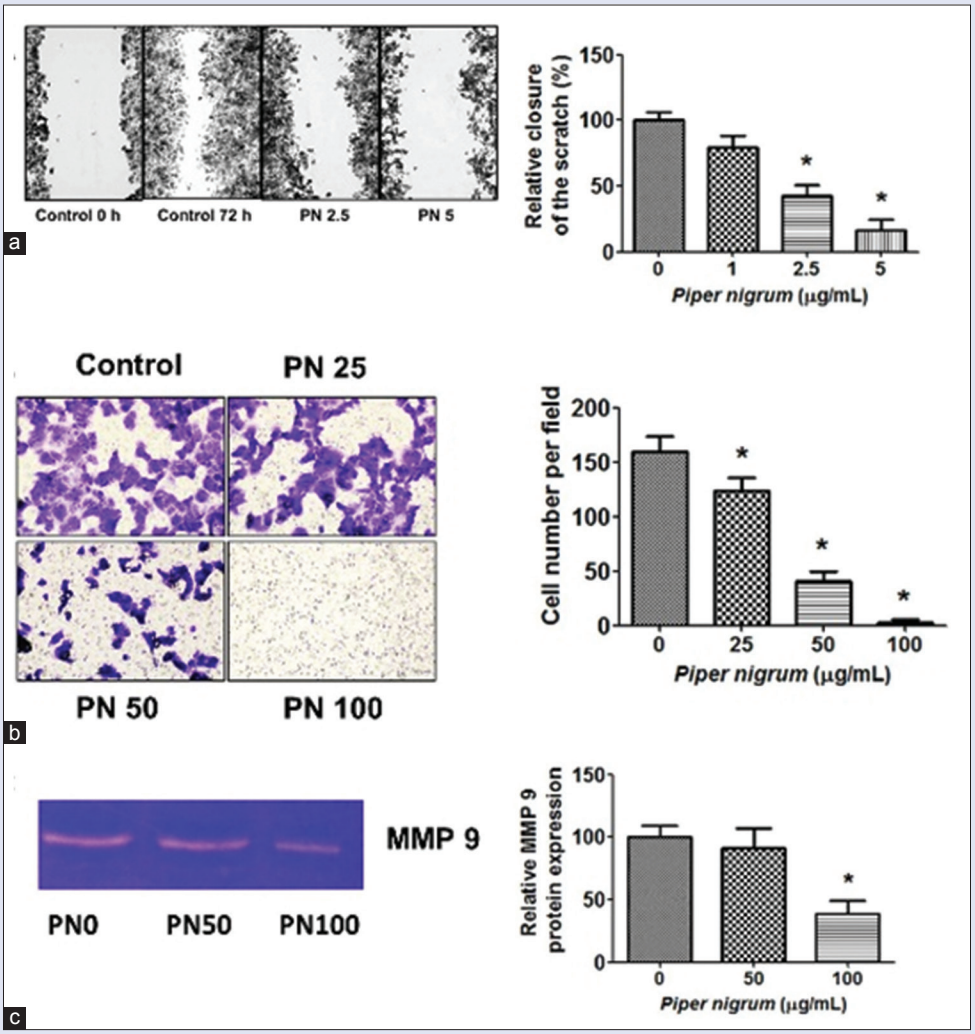


Figure 5: Effects of PN extract on cell migration of breast cancer cells (a-c). Cells were treated with 0–5 μg/mL for 72 h and examined the wound healing assay. Cells were treated with 0–100 μg/mL for 24 h and examined the Transwell chamber assay. Cells were treated with 0–100 μg/mL for 48 h and examined the gelatin zymography for detecting MMP 9 level. Data represent mean ± standard error of the mean of three independent experiments. *$P < 0.05$ versus control. PN: *Piper nigrum*

inhibition of the MCF-7 cell growth ability after treated with PN extracts was consisted with the cancer cells arrest at G1 phase along with an accompanied reduce in the S phase. Furthermore, the anti-proliferative mechanism of the PN extract at the molecular and cellular levels was investigated. The PN extract induced ROS formation at the significant level of 500 μg/mL. Similarly, de Souza Grinevicius *et al.* showed that a PN ethanolic extract has a high level of piperamides caused ROS over-production in MCF-7 cells at approximately 65%,[5] induced oxidative stress damage in DNA and then stimulated cell cycle arrest and caused cancer cells apoptosis. Further, the PN extract activated the caspase-3 activity accompanied by caspase-3 protein expression. Moreover, the PN extract reduced cyclin D1 and NF-κB protein expression levels. PN or piperine derivative, pipernonaline, increased the intracellular Ca^{2+}, reduced mitochondrial function, and increased cancer cell apoptosis by activating caspase-3 and down-regulating PARP cleavage, but do not interfere with bax and bcl-2 level regulation.[8] Our results suggest that the mechanism of actions of PN extract on the growth inhibitory effect may be through cell cycle arrest and apoptosis induction in breast cancer cells.

The metastasis of cancer cells is a basic mechanism of cancer cells to migrate to distant organs.[23] Truly, a herbal medicine with the capability to inhibit the metastasis could be a potential candidate for preventing and treating cancer. MMP 2 and MMP 9 are a crucial role in many cancer steps, for example, invasion, migration, metastasis, and tumorigenesis.[24,25] These studies indicated that PN extracts suppressed the basal activity of migration of MCF-7 cells through inhibiting wound healing, matrigel migration, and gelatin zymography assay. Consistency with PN reduced the down-regulation of metastasis-associated genes expression, including MMP 2, MMP 9, VEGFA, and ICAMP1. Surprisingly, the PN extract had the greatest effects on VEGFA and ICAMP1. Our results correlate with Senggunprai *et al.* in which the plant extract inhibited cholangiocarcinoma cell migration by suppressing MMP 9, ICAMP, VEGF, iNOS, and COX 2.[15] It should be noted that the anti-metastatic effect of the ethanolic extract of PN was discovered at lower concentrations than growth inhibition, which minimally decreased cell migration. Our results indicate that the PN extract may probably exert this migratory effect through suppression of MVP activation.

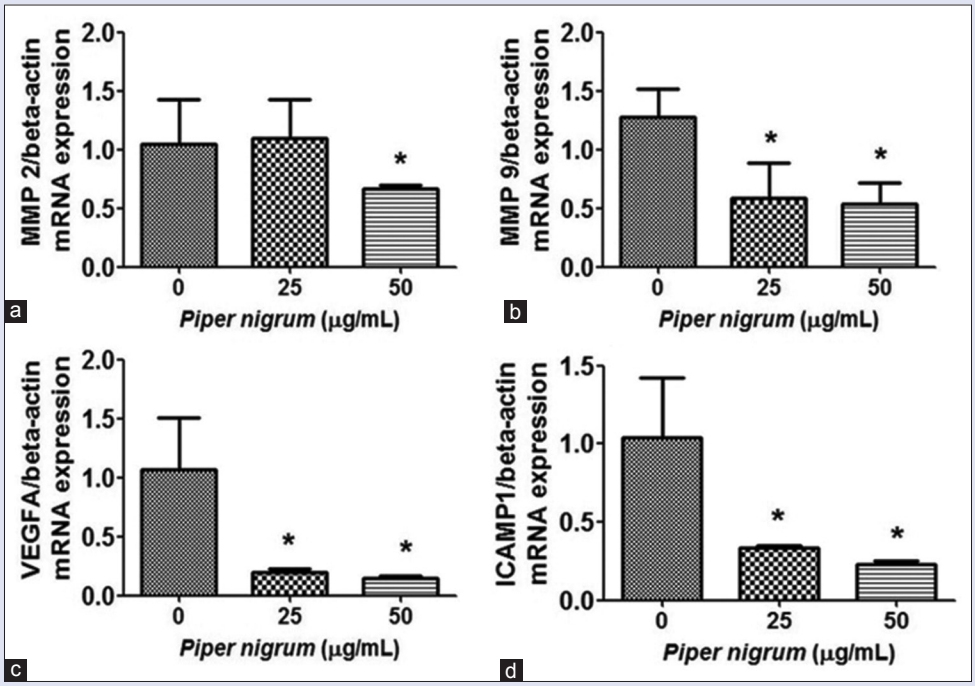


Figure 6: Effects of PN extract on gene expression of MMP 2, MMP 9, VEGFA, and ICAMP1 in breast cancer cells (a-d). Cells were treated with 0–50 µg/mL for 24 h and then examined gene expression using reverse transcription polymerase chain reaction. Data represent mean ± standard error of the mean of three independent experiments. *$P < 0.05$ versus control. PN: *Piper nigrum*

CONCLUSION

The PN extracts exhibited capable anti-growth activity defeat human breast cancer cells through inducing cell cycle arrest and activating apoptosis contributing in part to their reduced proliferation and reduced migration. In addition, the PN extract that contains piperine could serve as a promising anti-cancer agent for human breast cancer development by obstructing MVP pathways. PN could be provided a new natural compound to inhibit MCF-7 cells growth and migration through MVP pathway.

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Conflicts of interest

There are no conflicts of interest.

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