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In Vitro anti-metastasis of *Perilla frutescens* leaf water extract on aggressive human breast cancer cells

Chakkrit Khanaree[1] Wanisa Punfa[1] Payungsak Tantipaiboonwong[2] Piyawan Nuntaboon[2] Maitree Suttajit[2] Supachai Topanurak[3] Nahathai Dukaew[4, 5] May Thuu Mon[6] Rentong Hu[7] Komsak Pintha[2*]

[1]School of Traditional and Alternative Medicine, Chiang Rai Rajabhat University, Chiang Rai Province, Thailand
[2]Division of Biochemistry, School of Medical Sciences, University of Phayao, Phayao Province, Thailand
[3]Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
[4]Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai Province, Thailand
[5]Clinical Research Center for Food and Herbal Product Trials and Development (CR-FAH), Faculty of Medicine, Chiang Mai University, Chiang Mai Province, Thailand
[6]Department of Biochemistry, University of Medicine 1, Yangon, Myanmar
[7]Department of Laboratory Medicine, The Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, Guangxi, China

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ABSTRACT

Background: *Perilla frutescens* is a long-established plant that is often used in foods and traditional medicines in Asian countries. The perilla leaf contains a considerable number of bioactive substances, such as phenolics and flavonoids, which have been demonstrated to possess anticancer activity *in vitro* and *in vivo*.

Objectives: We aimed to study anti-metastatic activity, anti-invasion activity, and anti-migration activity of perilla leaf water extract (PLW) at 90°C for 1-5 min in MDA-MB-231 aggressive human breast cancer cells.

Materials and methods:Dry perilla leaves were extracted using hot water for 1-5 min to obtain crude extract and then lyophilized for PLW powder. PLW was evaluated for total phenolic, total flavonoid, and rosmarinic acid (RA) contents by Folin-Ciocalteau reagent, aluminum chloride colorimetric assay, and ultra-high-pressure liquid chromatography, respectively. Antioxidant activity of PLW was determined by DPPH and ABTS assays. MTT assay was performed to evaluate the cytotoxicity of PLW on MDA-MB-231 cells. Effective PLW was further determined its inhibitory effect on human breast cancer cell metastasis by a Boyden chamber-based transmembrane assay, the MMP-9 activity, and the proteolytic type IV collagenase activity.

Results: PLW by 5-min infusion showed the highest amount of total phenolic and flavonoid contents, as well as RA. Moreover, by the 5-min infusion, PLW had the highest antioxidant capacity when compared to PLW by infusions for 1-4 min. Following that, cytotoxicity testing revealed that the PLW is not toxic to MDA-MB-231 cells after a 24-hr exposure. The PLW at non-toxic doses (12.5-100 µg/mL) intensely presents an inhibitory effect on cell invasion and migration. The gelatinolytic activity showed that the PLW at concentrations of 12.5-100 µg/mL decreases MMP-9 activity in a dose-related manner. Furthermore, after treatment with the PLW, the proteolytic type IV collagenase activity was reduced considerably in a dose-related manner.

Conclusion: Our findings further showed that the PLW samples inhibit proteolytic enzymes involved in basement membrane breakdown, which might explain the anti-invasion and anti-migration properties of breast cancer cells. From the result, the application of perilla leaf might be developed as an herbal tea and used as an anti-metastatic agent for breast cancer prevention and treatment.

* **Corresponding author.**
Author's Address: *Division of Biochemistry, School of Medical Sciences, University of Phayao, Phayao Province, Thailand.*
E-mail address: komsakjo@gmail.com
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Introduction

Female breast cancer is the most commonly diagnosed cancer and was the fifth leading cause of cancer death globally, in 2020.[1] In Thailand, breast cancer is the third most commonly occurring cancer and the third leading cause of cancer death.[2] Importantly, metastasis of breast cancer cells remains the primary cause of death in the majority of breast cancer patients.[3] The breast cancer cell metastasis occurs as the common metastatic process seen in a variety of solid tumors, which is promoted by the crucial steps including detachment from the primary tumor, migration, invasion, and travel to other sites through blood and lymphatic vessels, then adhesion and growth at a site other than the primary tumor.[4] The crucial step that promotes cancer cell metastasis happens through the activity of proteolytic enzymes, particularly matrix metalloproteinases (MMPs) and collagenase, on the extracellular matrix (ECM). Especially, the type IV collagen-specific collagenases and MMP-9, show an important role in the degradation of ECM components.[5,6] Inhibiting ECM-degrading enzymes is thus an attempt to prevent the metastasis of the cancer cells.

Recently, various investigations have provided support for the use of plant phytochemicals and derivatives in cancer metastasis prevention. Interestingly, several studies suggested that plant polyphenols, such as epigallocatechin gallate, resveratrol, curcumin, and rosmarinic acid can inhibit breast cancer cell metastasis by suppressing MMPs and collagenases activity.[7-10] Thus, plant polyphenols may offer a new source of anti-metastasis agents against breast cancer cells.

Perilla frutescens is used as functional foods and traditional medicines for the treatment of several conditions and diseases in Asian counties.[11] It is an aromatic plant of the mint family (Lamiaceae) that grows up to 4–6 feet tall with square and branching stems. Its dark green and hairy leaf is large (7x12 cm) with an oval shape and pointy end (Figure 1). Many studies have revealed that the Thai perilla leaf contains a variety of bioactive polyphenol substances such as rosmarinic acid, luteolin, and apigenin, which have a wide range of biological activities including anti-mutagenicity, anti-inflammation, anti-oxidant activities, and inhibition of carcinogenesis.[12] Moreover, our previous study showed that perilla leaf ethanolic extract (PLE) could inhibit migration and invasion of aggressive breast cancer cell line, MDA-MB-231.[13] Thus, perilla leaf has the potential to be developed as a herbal supplement for anti-metastasis agents against breast cancer.

However, ethanol, the most commonly effective solvent used for extracting bioactive compounds, is more toxic and regarded as an environmental pollutant. Unlike ethanol and organic solvents, water is an environmentally friendly solvent. It is a nontoxic, nonflammable, and simple solvent.[14] Using the different solvents and extraction processes might be different in bioactive substances and their activities. A study in 2018 by Pintha *et al*. showed that PLE by means of maceration has anti-metastatic properties *in vitro*.[13] In the current study, we explored the anti-metastatic activities including anti-invasion and anti-migration of perilla leaf water extract (PLW), which was prepared by means of infusion and compared with PLE.

Materials and methods

Plant material and extraction

Thai perilla leaf samples were collected from Phayao, Thailand. The voucher number was deposited at Queen Sirikit Botanic Garden Herbarium, Chiang Mai, Thailand (Code: QBG-93756). For the preparation of PLW, the dried leaves of perilla were firstly finely crushed and infused in hot water (90°C) for 1-5 min. Then, the extracts were filtered through filter paper (Whatman No.1), lyophilized, and powdered. For the preparation of PLE, the dried leaves of perilla were extracted with 70% ethanol at a 1:10 ratio for 12 hrs with constant stirring and then left overnight. The ethanolic solution was then filtered using filter paper (Whatman No.1). The PLE was evaporated and lyophilized to powder, respectively. PLW and PLE were kept at -20°C until used for further experiments.

Quantification of total phenolic content (TPC)

Briefly, 20 µL of the different concentrations of PLW or PLE dissolving in DMSO (RCI Labscan limited, Bangkok, Thailand) was added to 100 µL of Folin-Ciocalteu reagent (10% v/v) (Merck, Darmstadt, Germany) into 96-well plate and incubated in the dark standing at room temperature. After 3 min, 80 µL of sodium carbonate (7.5% w/v) (VWR Chemicals BDH®, Leicestershire, UK) was added. The reaction was incubated for 30 min at room temperature in the dark, the absorbance was measured at 765 nm using a Synergy™ HT Multi-Detection Microplate Reader (BioTek Instruments, Inc., VT, USA). TPC was expressed in milligram of gallic acid equivalents (GAE) per gram dry weight of extract (mg GAE/gm extract) using a calibration gallic standard curve.[15]

Quantification of total flavonoid content (TFC)

Concisely, 25 µL of the assigned concentrations of PLW or PLE in DMSO (RCI Labscan limited, Bangkok, Thailand) was mixed with 125 µL of deionized water and 7.5 µL of 5% $NaNO_2$ (VWR Chemicals BDH®, Leicestershire, UK) in a 96-well plate. Following 6 min, 15 µL of 10% (w/v) aluminum chloride (VWR Chemicals BDH®, Leicestershire, UK) was added to the mixture at room temperature in the dark for another 6 min. After that, 50 µL of 1 M NaOH (VWR Chemicals BDH®, Leicestershire, UK) was added and the volume was made up to 250 µL with deionized water. The reaction was incubated at room temperature in the dark for 15 min and the absorbance of sample was measured at 532 nm using a Synergy™ HT Multi-Detection Microplate Reader (BioTek Instruments Inc., VT, USA). TFC was expressed in milligrams of catechin equivalents (CE) per gram dry weight of extract (mg CE/gm extract) using a calibration catechin standard curve.[15]

Detection of rosmarinic acid (RA) in PLW and PLE

The ultra-high-pressure liquid chromatography (UHPLC) condition was performed using reverse-phase ZORBAX Eclipse plus C18 column (4.6 mm x 150 mm, 5 µm particle diameters) (Agilent Technologies Inc., CA, USA). The assay was performed using two solvents, including 0.1% trifluoroacetic acid (VWR Chemicals BDH®, Leicestershire, UK) in acetonitrile and 100% methanol. Samples (10 µL) were injected into the column with a flow rate of 1.0 mL/min. The RA peaks were detected at 280 nm. The peak area and retention time of each fraction were compared with a calibration curve of

various concentrations of RA standard and calculated and expressed as mg/gm extract.[16]

Determination of antioxidant activity by DPPH radical scavenging assay

In brief, the mixture of 20 µL of assigned concentrations PLW or PLE and 180 µL of 0.2 mM DPPH reagent (Sigma-Aldrich, MA, USA) were added to a 96-well plate. After incubation at room temperature in the dark for 30 min, absorbance of the reaction mixture was detected at 517 nm against blank as methanol using a Synergy™ HT Multi-Detection Microplate Reader (BioTek Instruments Inc., VT, USA). The percentage of scavenging inhibition was calculated, and the antioxidant activity was expressed as scavenged free radicals by 50% (SC_{50}).[17]

Determination of antioxidant activity by ABTS radical scavenging assay

Briefly, the mixture (10 µL) of the PLW or PLE assigned concentrations and 990 µL of working ABTS solution (Sigma-Aldrich, MA, USA) were incubated for 6 min at room temperature in the dark. The absorbance of the reaction sample was detected at 734 nm against with distilled water as blank reference, using the UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). The percentage of scavenging inhibition was calculated and the antioxidant activity was expressed as scavenged free radicals by 50% (SC_{50}).[17]

Cell lines and culture condition

MDA-MB-231 human breast carcinoma cells and NIH3T3 fibroblast cells were obtained from the American Type Culture Collection (ATCC, DC, USA). MDA-MB-231 and NIH3T3 cell lines were cultured and maintained in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific Inc., MA, USA) supplemented with 10% (v/v) fetal bovine serum (Thermo Fisher Scientific Inc., MA, USA), penicillin (100 U/mL) and streptomycin (100 µg/mL) (Thermo Fisher Scientific Inc., MA, USA) in 5% CO_2 with humidified incubator at 37°C.

Determination of cytotoxicity by MTT assay

The MDA-MB-231 cells were seeded in a 96-well plate for 24 hrs prior treatment with PLW or PLE (0-200 µg/mL) for 24 hrs. Next, 15 µL of MTT dye (5 mg/mL) (AppliChem, Darmstadt, Germany) was added and incubated at 37°C for 4 hrs. After 4 hrs, all solutions were removed. Then, DMSO (100 µL) (RCI Labscan limited, Bangkok, Thailand) was added to dissolve the formazan crystal. The formazan crystal product measured the absorbance at 540/630 nm using a Synergy™ HT Multi-Detection Microplate Reader (BioTek Instruments Inc., VT, USA). The percentage of cell viability was calculated and non-cytotoxic concentrations ($\leq IC_{20}$) were used for the further experiments.[15]

Determination of cell migration and invasion

Cell migration was tested using polyvinylpyrrolidone-free polycarbonate filters (Merck, Darmstadt, Germany) coated with 0.01% (w/v) gelatin (Sigma-Aldrich, MA, USA), whereas cell invasion was tested with a filter coated with Matrigel (Corning, NY, USA) (15 gm per filter). The NIH3T3 fibroblast cell culture media was added to the lower chamber to function as a chemoattractant. The top inserts were then seeded with 1.5×10^5 MDA-MB-231 cells in DMEM containing various doses of PLW or PLE (0-100 µg/mL). After that, the chambers were incubated at 37°C with 5% CO_2. Cells that migrated or invaded through the bottom surface of the membrane were preserved with methanol and stained with toluidine blue (VWR Chemicals BDH®, Leicestershire, UK) after an 18 hrs incubation period. Migrating or invading cells in 20% acetic acid were detected at the 570 nm absorbance with a Synergy™ HT Multi-Detection Microplate Reader (BioTek Instruments Inc., VT, USA). The cell migration or invasion were calculated as follows: $(OD_{treated}/OD_{control})\times100\%$.[18]

Determination of MMP-9 activity

The culture supernatant of MDA-MB-231 cells after treatment with PLW or PLE (0-100 µg/mL) was subjected to gel electrophoresis. Equal quantities of total protein of each treatment were loaded on 10% polyacrylamide gels (Bio-Rad, CA, USA) containing 0.1% (w/v) gelatin (Bio-Rad, CA, USA). The electrophoretic gels were sliced into single-lane strips after being washed twice with Triton X-100 (AppliChem, Darmstadt, Germany). Each gel strip was re-incubated in activation buffer for 24 hrs with PLW or PLE (0–100 µg/mL) and then stained with 0.1% (w/v) Coomassie Brilliant Blue R (Bio-Rad, CA, USA). The proteolytic activity of MMP-9 was thought to be displayed as clear bars on a blue backdrop, signifying digested bands. The digested bands were quantified using Bio 1D software (Viber Lourmat).[16]

Determination of type IV collagenase activity

An EnzChek collagenase assay kit (Thermo Fisher Scientific Inc., MA, USA) was used to determine the proteolytic activity of type IV collagenase (Molecular Probe). In a 96-well microplate, 1 U/mL collagenase was combined with 10 µg/mL fluorescein-conjugated gelatin (DQ gelatin) with different doses of PLW or PLE suspended in reaction buffer. The rate of proteolysis was measured by using a fluorometer to measure fluorescence intensity at 3 min intervals for 30 min. At an excitation wavelength of 485 nm and an emission wavelength of 528 nm, the fluorescence levels were measured. The activity of enzyme inhibitors was calculated using linear regression of the fluorescence intensity obtained at the time.[16]

Statistical analysis

The results were presented as mean±standard deviation of three independent experiments using one-way ANOVA with Dunnett's test. For all assessed multiple comparisons, Prism version 6.0 software was utilized, and $p<0.05$, $p<0.01$, and $p<0.001$ were measured to be significant.

Results

Phenolic and flavonoid contents of PLW compared with PLE

Table 1 shows the total phenolic and flavonoid contents of PLW at different times of infusion (1–5 min) compared to PLE. The TPC of the PLW (infusion for 1-5 min) ranged from 195-222 mg GAE/gm extract, whilst the TFC of the PLW (infusion for 1-5 min) ranged from 107-120 mg CE/gm extract. PLW by 5-min infusion exhibited greater TPC (222±2.73 mg GAE/gm extract) and TFC (120±4.41 mg CE/gm extract) as compared to PLW by infusion for 1-4 min. However, the greatest amounts of TPC (260±15.24 mg GAE/gm extract) and TFC (136±6.59 mg CE/gm extract) were found in PLE.

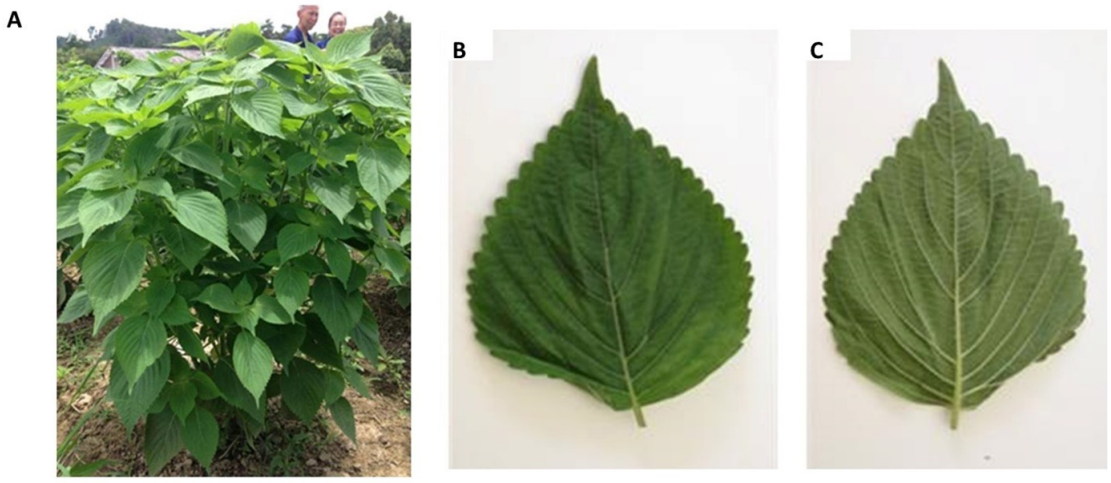

Figure 1. *Perilla frutescens plant (A), front side (B), and backside (C) of its leaves (Photos taken at University of Phayao, Thailand).*

Table 1 Total phenolic and flavonoid contents of PLW and PLE.

Extracts	%Yield	Total phenolic content (mg GAE/gm extract)	Total flavonoid content (mg CE/gm extract)
PLW by 1-min infusion	14.7	195.00±4.03	107.00±4.98
PLW by 2-mininfusion	15.6	204.00±1.86	112.00±7.80
PLW by 3-min infusion	14.5	205.00±0.89	115.00±6.26
PLW by 4-min infusion	15.3	206.00±4.73	116.00±6.28
PLW by 5-min infusion	15.4	222.00±2.73	120.00±4.41
PLE	12.3	260.00±15.24*	136.00±6.59*

**p<0.05 vs PLW by infusion for 1-5 min.*

RA content in PLW compared to PLE

The main peak in the RA standard was observed at a retention time of 5.483 min (Figure 2G). The RA content in PLE was 167.54±0.92 mg/gm extract (Figure 2F). The RA contents in PLW by infusion for 1-5 min were 104.26±3.28, 105.60±0.69, 106.77±1.69, 108.56±0.10, and 110.23±1.66 mg/gm extract, respectively (Figures 2A-E). As shown in Figure 2, the PLW by 5-min infusion had the greatest amount of RA, when compared to the PLW by infusion for 1-4 min.

Antioxidant capacities of PLW and PLE

Table 2 showed the SC_{50} values of DPPH and the $ABTS^{\bullet+}$ scavenging radicals of PLW and PLE. For the DPPH method, SC_{50} values of PLW by infusion for 1-5 min ranged from 23.99-24.79 µg/mL. PLW by 5-min infusion showed the highest activity with the SC_{50} values of 23.99±0.31 µg/mL but did not show a significant difference when compared to PLE (23.26±0.76 µg/mL). Similarly, PLW by infusion for 1-5 min could inhibit the stable $ABTS^{\bullet+}$ radicals with SC_{50} values of 7.70-8.26 µg/mL, with the greatest activity as shown in PLW by 5-min infusion (7.70±0.14 µg/mL). On the other hand, PLE revealed the most $ABTS^{\bullet+}$ radicals' inhibitory activity with the SC_{50} values of 6.90±0.11 µg/mL that showed a statistical significance when compared to PLW by infusion for 1-5 min.

Cytotoxicity of PLW to MDA-MB-231 cells

MDA-MB-231 cells were treated with varying concentrations of the extracts (12.5-200 µg/mL) for 24 hrs and compared to non-treated cells to assess the cytotoxic impact of PLW by 5-min infusion and PLE. As demonstrated in Figures 3A-B, PLW by 5-min infusion and PLE at concentrations of 12.5-200 µg/mL did not cause substantial toxicity in MDA-MB-231 cells, when compared to untreated control cells. As a result, subsequent tests utilized PLW by 5-min infusion or PLE concentrations of up to 200 µg/mL. From the UHPLC results of this study, 1 gm of PLW by 5-min infusion yielded RA about 110 mg. Therefore, PLW by 5-min infusion at doses of 12.5-200 µg/mL was RA about 1.38-22 µg/mL. Thus, the effect of RA (1-20 µg/mL) on the viability of MDA-MB-231 cells was determined. There was no significant effect on cell viability after RA treatment for 24 hrs as shown in Figure 3C.

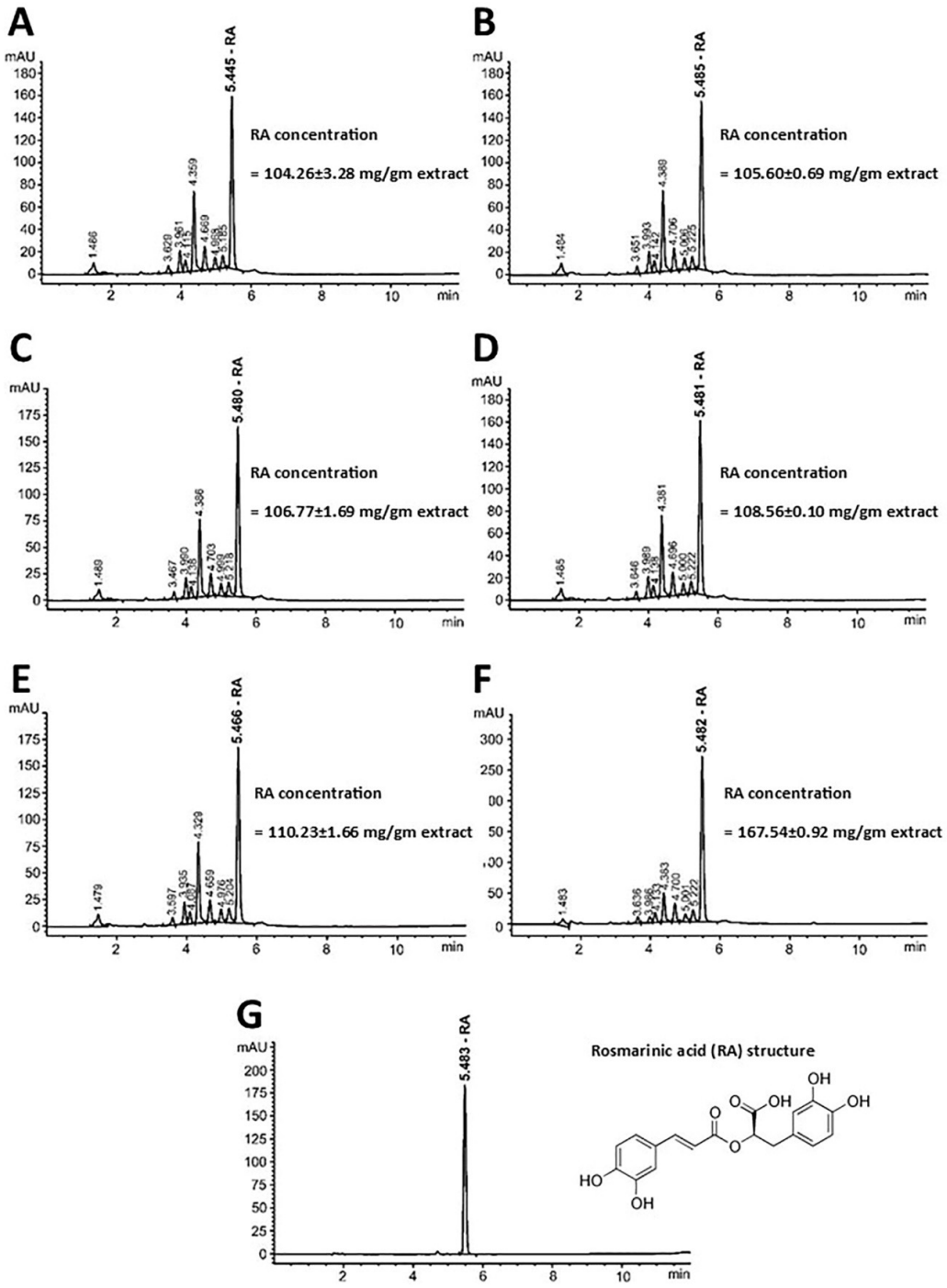

Figure 2. *The UHPLC chromatogram of PLW by 1-min (A), 2-min (B), 3-min (C), 4-min (D), and 5-min (E) infusion comparison with PLE (F) and RA standard (G).*

Table 2 The scavenge DPPH and ABTS$^{\bullet+}$ radicals of PLW and PLE.

Extracts	SC_{50} values of DPPH scavenging (µg/mL)	SC_{50} values of ABTS$^{\bullet+}$ scavenging (µg/mL)
PLW by 1-min infusion	24.57±0.37	8.19±0.32
PLW by 2-min infusion	24.48±0.63	8.26±0.25
PLW by 3-min infusion	24.79±0.38	7.75±0.20
PLW by 4-min infusion	24.72±0.21	7.79±0.12
PLW by 5-min infusion	23.99±0.31	7.70±0.14
PLE	23.26±0.76	6.90±0.11*

**p<0.05 vs PLW by infusion for 1-5 min.*

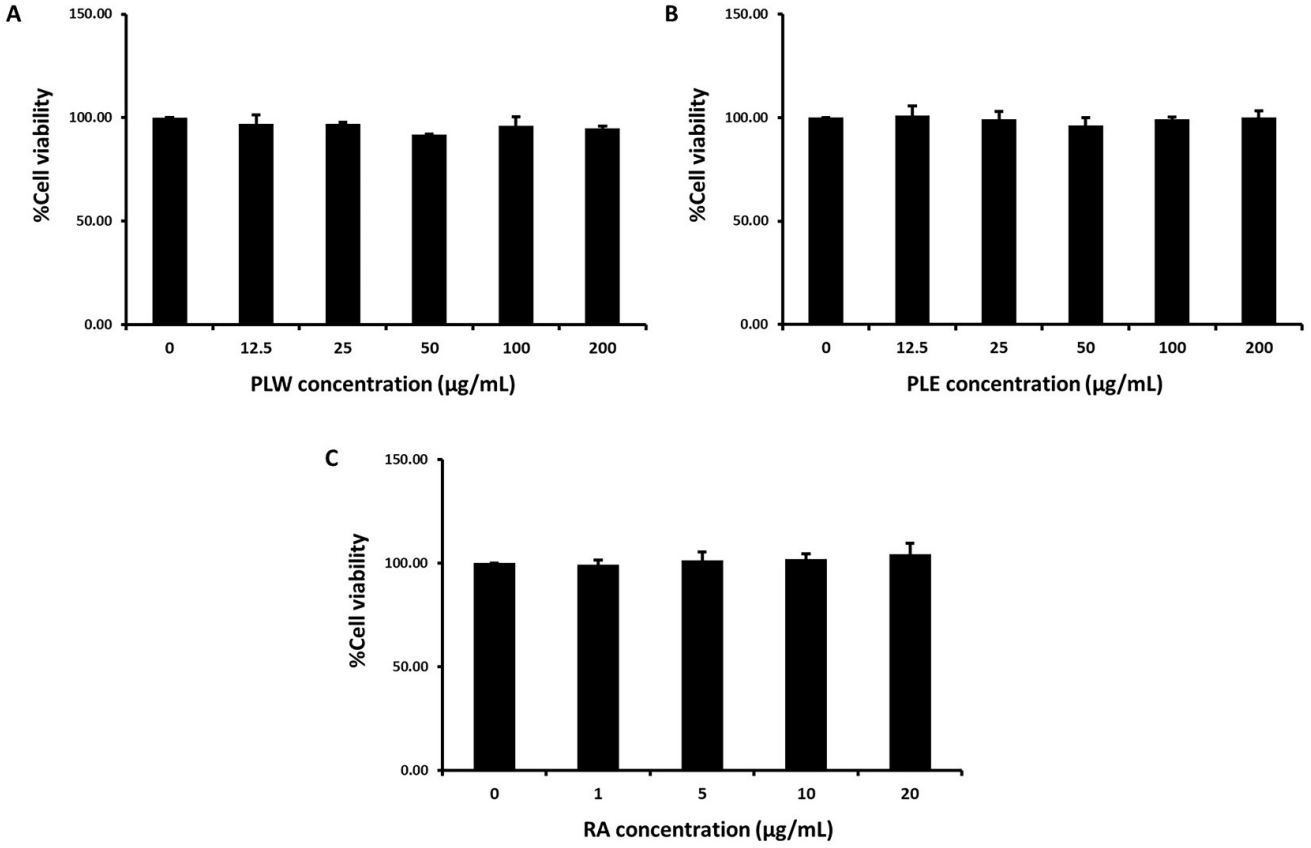

Figure 3. *The cytotoxic effect of PLW by 5-min infusion (A), PLE (B), and RA (C) on MDA-MB-231 cells. Data were represented as a percentage of viable cells compared to the untreated control cells.*

Inhibition effect of MDA-MB-231 on cell migration and invasion by PLW

MDA-MB-231 cells were shown to have considerably less invasion and migration when exposed to PLE at a concentration of 100 µg/mL (Figure 4). As shown in Figure 4A, PLW by 5-min infusion concentrations of 12.5-100 µg/mL decreased cell invasion of MDA-MB-231 cells passing through the Matrigel in a dose-related manner with IC_{50} values at 71.03±2.82 µg/mL. On the other hand, PLW considerably reduced cell migration when tested on the gelatin-coated filters (Figure 4B) as well as PLE (100 µg/mL). In addition, MDA-MB-231 cells were treated with RA at a non-toxic dose of 10 µg/mL. At this concentration, RA remarkably decreased the cell migration and invasion by approximately 50% and 30%, respectively when compared with the untreated cells. Our results confirmed that RA in PLW can evidently reduce MDA-MB-231 cell invasion and migration.

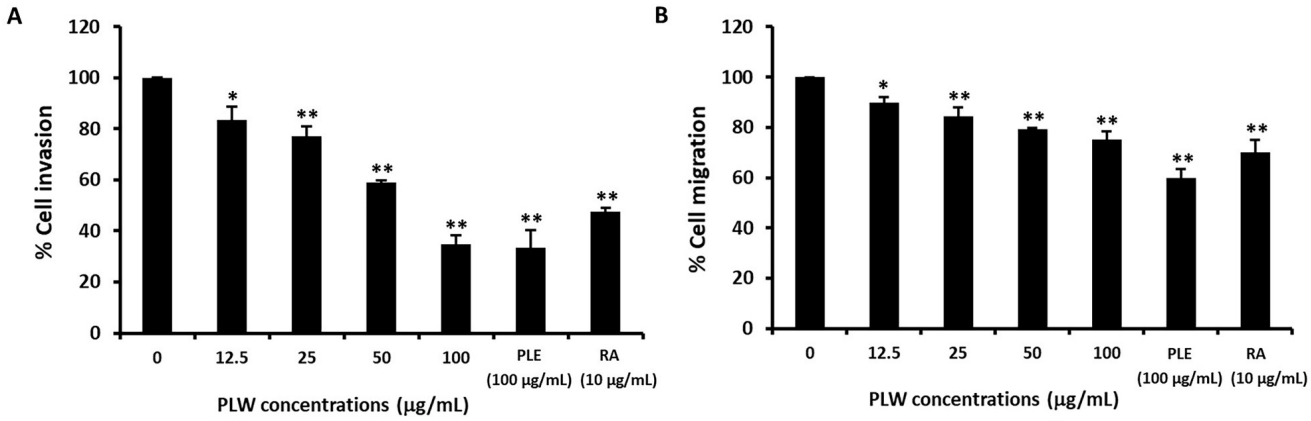

Figure 4. *Effect of PLW by 5-min infusion, PLE, and RA on cell invasion and cell migration in MDA-MB-231 cells. Cell invasion (A) and cell migration (B) were expressed as a percentage compared to the untreated control cells. (*p<0.01, **p<0.001 when compared to untreated control cells).*

Reduction of activity of MMP-9 by PLW

The different doses of PLW by 5-min infusion (0-100 µg/mL) or PLE (100 µg/mL) or RA (100 µg/mL) were added to the culture supernatant of MDA-MB-231 cells to determine whether the extracts might decrease MMP-9 activity. The results demonstrated that the administration of PLW by 5-min infusion (25-100 µg/mL) dramatically reduces the activity of MMP-9 in a dose-related manner (Figure 5A). Moreover, MMP-9 activity is substantially reduced by treatment with PLE (100 µg/mL) or RA (100 µg/mL).

Inhibition activity of type IV collagenase by PLW

Collagenase was incubated with varied concentrations (0-100 µg/mL) of PLW by 5-min infusion to assess the inhibitory effect of the PLW on the activity of type IV collagenase. The results demonstrate that PLW by 5-min infusion, as well as PLE (100 µg/mL) and RA (100 µg/mL), dramatically decrease type IV collagenase activity in a dose-related manner (Figure 5B).

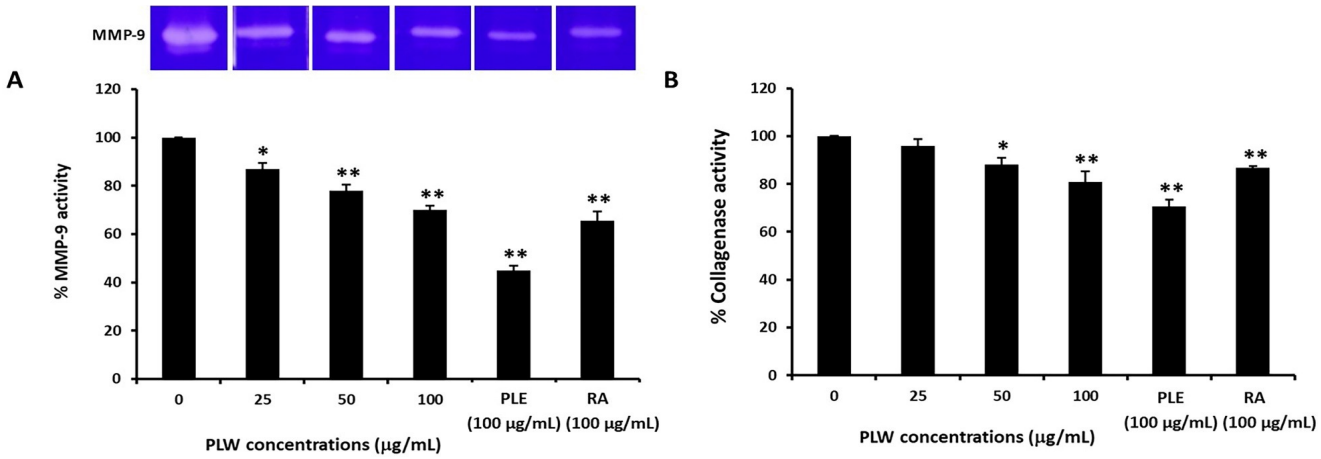

Figure 5. *Effects of PLW by 5-min infusion, PLE, and RA on MMP-9 and type IV collagenase activities. (*p<0.01, **p<0.001 when compared to untreated control cells).*

Discussion

The therapeutic options to prevent metastasis in breast cancer patients are currently limited. Standard chemotherapeutics mainly exert anti-cancer effects by mechanisms such as inhibiting DNA/RNA synthesis, inducing apoptosis or suppressing cell proliferation, although they do not prevent cell motility. As a result, cancer cells that survive or resist chemotherapy can undergo migration and invasion, resulting in metastases.[19] The search for new anticancer agents with anti-metastatic effects is still therefore required.

Perilla frutescens is an herbal medicine regularly used and cultivated in nations throughout South-East Asia.[11] It has been demonstrated to contain polyphenol compounds such as rosmarinic acid, apigenin, and luteolin, which have a wide range of biological activities. Our previous study showed that PLE by mean of maceration has antimetastatic properties *in vitro*.[13] However, ethanol might be regarded as environmental pollutant. In certain processes, water can be replaced ethanol for food and pharmaceutical manufacturing, resulting to reducing costs and eliminating the environmental problems.[14] Thus, this study aimed to investigate the development of perilla leaf extract used as herbal tea supplement for anti-metastasis of breast cancer. To extract bioactive compounds from perilla leaf, this study utilized hot water infusion and ethanol maceration. The results showed that different extraction methods, solvents, and times caused different contents of bioactive compounds in the extracts. Moreover, PLE has been shown to contain both polar and non-polar compounds, whereas PLW contains only polar compounds. However, this study found that RA was the main component and bioactive ingredient in PLW and PLE. RA is a phenolic compound which has been used as a natural food supplement. There are many reports about the beneficial activities on health, including antioxidant, anti-microbial, anthelmintic, anti-inflammatory agents, anti-allergy, anti-diabetic, and neuroprotective functions.[20] For anti-cancer effects, RA could suppress the development of cancers in several organs including the colon, breast, liver, stomach, as well as leukemia cells.[21]

Extraction solvents and time have an impact on bioactive components, which, in turn, have a main effect on the extract's biological activity.[22, 23] The antioxidant activity of extracts was investigated in this study utilizing the DPPH and $ABTS^{\bullet+}$ scavenging radical activity assays. The results suggested that PLW still had antioxidant activity as well as PLE, with the maximum activity being PLW by 5-min infusion. Consequently, PLW by 5-min infusion was utilized in the subsequent studies and further characterization of its anti-metastasis activity was compared to PLE.

Invasion and migration of cancer cells are crucial components of cancer metastasis, which is the major cause of cancer recurrence and death in cancer patients. Even though breast cancer therapies have advanced to unprecedented levels in recent years, many patients continue to face the problem that their cancer's proliferation and metastasis cannot be managed, partially due to inadequate therapy or therapeutic resistance.[3] The results represent the inhibitory efficiency of PLW against migration and invasion of breast

cancer cells. Similarly, the previous reports of Cho-Long Kim revealed that PLE could decrease cell migration and invasion in breast cancer cells.[24] Moreover, RA, the major compound presented in PLW, inhibited the migration of derived breast cancer stem-like cells, and inhibited the invasion of MDA-MB-231.[13, 25] Thus, the RA in PLW supports the anti-migration and anti-invasion effects, which reflect the anti-metastatic activity against breast cancer.

To better understand its anti-metastatic characteristics, the inhibitory effects of PLW on the proteolytic enzymes involved in ECM breakdown were investigated. ECM degradation by MMPs is a crucial stage in tumor invasion.[26] The deregulation of MMP expression stimulates invasion and metastasis in breast cancer.[27] Many studies have suggested that MMP-9 is involved in the development of invasive breast cancer through the metastatic cascade, as well as being a potential prognostic factor.[5] In addition, Type IV collagenase, one of the most significant members of the MMPs family, has been shown to be intimately connected with many tumor systems and linked to tumor cell invasive potential.[6, 28] Type IV collagenase overexpression has been detected in a number of malignancies, including colorectal cancer, gastric cancer, and breast cancer.[29] This demonstrates the ability of the PLW and PLE to inhibit MMP-9 and type IV collagenase activities. According to RA, a key component of PLW, was shown to reduce MMP-9 production in an animal model by activating AMPK and inhibiting its directly and competitively.[30]

The new findings of this study showed that perilla leaf can be extracted by water. Furthermore, PLW has an anti-metastatic activity in breast cancer cells. However, the effects of PLW have not been previously studied and reported. Most experiments used ethanol or methanol as the solvent for extraction. These toxic solvents must be eliminated before consumption or use in humans. The solvent-free extracts are important and necessary for the development of herbal supplements or herbal drugs. Therefore, the Thai perilla leaf has a vital potential for establishment as an instant drink, functional tea, and herbal tea for cancer metastasis prevention and treatment.

Conclusion

In this study, we demonstrated that PLW can suppress the migration and invasion in aggressive breast cancer cells. PLW works by reducing the space available for cell movement through inhibiting MMP-9 and type IV collagenase activities, which is a crucial step of metastasis. These findings indicated that perilla leaf extract, infused by hot water, inhibits proteolytic enzymes involved in ECM breakdown, reducing the process of breast cancer cell migration and invasion. Therefore, the applications of perilla leaf might also be developed as an instant drink, functional tea, and herbal tea for anti-metastatic breast cancer treatment.

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Conflicts of interest

There are no conflicts of interest.

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