RESEARCH ARTICLE

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Atractylodes lancea Crude Extract Suppresses Triple-Negative Breast Cancer Metastasis via NF-kB signaling Pathway Inhibition

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Abstract

Objective: Triple-negative breast cancer (TNBC) is an aggressive subtype characterized by poor prognosis, high recurrence rates, and limited targeted therapies, often leading to metastasis in the brain, bones, lungs, and liver. The NF-kB signaling pathway plays a crucial role in TNBC metastasis. However, the potential of novel herbal medicines as anti-metastatic agents remains underexplored. This study investigates the effects of *Atractylodes lancea* (AL) crude extract on phosphorylated p65 NF-kB reduction and its impact on TNBC cell migration and invasion. **Methods:** High performance liquid chromatography (HPLC) analysis identified bioactive compounds in AL crude extract. Cytotoxicity was evaluated using MTT assays on MDA-MB-231 cells at 24 and 48 hours. Sub-lethal doses from cytotoxicity assays were used to assess anti-migration and anti-invasive effects via wound healing and gelatin zymography assays, respectively. Immunoblot analysis examined epithelial-mesenchymal transition (EMT)-related proteins and NF-kB expression. **Result:** AL crude extract significantly inhibited TNBC cell proliferation in a dose- and time-dependent manner, with IC_{50} values of 92.11±0.01 µg/ml (24 h) and 95.80±0.01 µg/ml (48 h). Wound healing assays confirmed reduced cell migration, while gelatin zymography showed decreased matrix metalloproteinase-9 (MMP-9) enzymatic activity. Immunoblot analysis revealed increased E-cadherin expression, reduced vimentin expression, and significant suppression of phosphorylated NF-κBp65 levels. **Conclusion:** AL crude extract exhibits promising anti-cancer effects by modulating EMT markers, reducing cell motility, and inhibiting NF-κB signaling. These findings suggest its potential as a therapeutic agent for TNBC metastasis suppression.

Keywords: Triple-negative breast cancer- *Atractylodes lancea*- Epithelial-mesenchymal transition

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Introduction

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by the absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) expression [1]. Due to the lack of targeted therapeutic options, TNBC is often diagnosed at an advanced stage, resulting in high recurrence rates and poor survival outcomes [2]. Metastatic TNBC commonly spreads to the brain, bones, lungs, and liver [3], further worsening patient prognosis. While several FDA-approved treatments for TNBC exist, their clinical efficacy is often limited by adverse effects, including cardiotoxicity, hypotension, and urinary tract infections [1].

Metastasis is a complex, multistep process that begins with increased cancer cell motility and invasiveness, primarily driven by epithelial-mesenchymal transition (EMT). EMT is a biological process in which epithelial cells lose their polarity and cell-cell adhesion properties,

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transitioning into a mesenchymal phenotype with enhanced migratory and invasive capabilities [4]. This transition is marked by the downregulation of epithelial markers, such as E-cadherin, and the upregulation of mesenchymal markers, including N-cadherin, vimentin, and fibronectin [5]. Additionally, tumor cells secrete matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, which degrade the basement membrane and extracellular matrix, facilitating invasion and metastasis. The NF-κB signaling pathway plays a crucial role in promoting EMT, cell survival, angiogenesis, and immune modulation in breast cancer [6]. Despite the potential of NF-κB inhibition as a therapeutic strategy, balancing efficacy and toxicity remains a significant challenge.

Natural compounds with anti-metastatic properties offer promising alternatives for cancer treatment due to their lower toxicity profiles. *Atractylodes lancea* (AL), commonly known as Khod-Kha-Mao, is a tuberous plant from the Asteraceae (Compositae) family, traditionally used in East Asian medicine for treating rheumatism, digestive disorders, night blindness, and influenza. Recent studies have highlighted the anti-cancer potential of AL extract and its key bioactive components, Atractylodin (Atr) and β-eudesmol (BE) in various cancers, including cervical, gastric, and liver cancer, as well as leukemia. Notably, BE has been shown to inhibit the migration of cholangiocarcinoma (CCA) cells [7] by suppressing EMT via the PI3K/AKT and p38MAPK pathways [4]. Additionally, Atr has been reported to induce oxidative stress-mediated apoptosis and autophagy in breast cancer cells (MCF-7) by inhibiting the PI3K/Akt/mTOR pathway [8], while BE has been found to suppress cell proliferation and induces ferroptosis in TNBC cells (MDA-MB-468 and MDA-MB-231) by modulating the MAPK signaling pathway [9].

Despite these findings, research on the effects of AL crude extract in breast cancer remains limited. This study aims to investigate the anti-cancer properties of AL crude extract, focusing on its ability to inhibit TNBC cell migration. We hypothesized that AL crude extract exerts its anti-migratory effects by suppressing EMT-related proteins and MMP activity through the inhibition of NF-kB signaling.

Materials and Methods

Cell cultures

Human triple-negative breast cancer (TNBC) cells (MDA-MB-231), ATCC (#HTB-26, Middlesex, UK) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc., MA, USA), 100 U/ml penicillin, and 100 mg/ml of streptomycin. The cells were maintained at 37°C in a humidified incubator with 5% CO2. The culture medium was replaced twice per week. For passaging, the cells were detached using 0.25% trypsin-EDTA solution and re-seeded into fresh culture medium.

Preparation of Atractylodes lancea crude extract and high-performance liquid chromatography analysis

Atractylodes lancea (AL) crude extract was provided by Professor Dr. Kesara Na-Bangchang from the Chulabhorn International College of Medicine, Thammasat University. The extraction process followed the methodology described by Na-Bangchang et al. [10]. To prepare the stock solution, the AL crude extract was dissolved in absolute ethanol to a final concentration of 70 mg/ml. High-performance liquid chromatography (HPLC) was used to analyze the bioactive components, comparing the composition of the extracted AL with standard reference compounds. The HPLC conditions were based on the method described by Jin Sun et al [11]. The mobile phase consisted of 0.2% phosphoric acid in ultrapure water and acetonitrile, with a flow rate of 1 ml/min and a total run time of 80 minutes. Detection was performed at two wavelengths: 208 nm for β-eudesmol (BE) and 340 nm for Atractylodin (Atr).

Cell viability assay

The cytotoxicity of *Atractylodes lancea* (AL) crude extract on MDA-MB-231 cells was evaluated using the MTT assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Aldrich, St. Louis, MO, USA). Cells were seeded at a density of 10,000 cells per well in a 96-well plate and incubated for 24 hours at 37°C in a humidified atmosphere of 5% CO_2. Following incubation, the cells were treated with AL crude extract at concentrations ranging from 0 and 160 µg/ml and incubated for either 24 or 48 hours. After treatment, MTT reagent was added to each well and incubated for 3 hours at 37°C. The culture supernatant was then removed, and dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Optical density (OD) was measured at 570 and 690 nm using a microplate spectrophotometer (BioTex Instruments, USA). Cell viability percentages and IC_{50} values (the concentration required to inhibit 50% of cell growth) were calculated using GraphPad Prism version 5.0.

Wound healing assay

To evaluate the effect of AL crude extract on cancer cell migration, a scratch assay was performed. MDA-MB-231 cells were seeded in 24-well plates at a density of 2x105 cells per well and incubated for 24 hours. After incubation, the culture supernatant was removed, and a scratch wound was created using a sterile 200-µl pipette tip. The cells were then treated with sublethal concentrations of AL crude extract (40, 60, and 80 µg/ml). Cancer cell migration was assessed by measuring the wound area at 0 and 24 hours using an inverted microscope. The extent of wound closure was analyzed with ImageJ software to quantify cell migration.

Gelatin zymography assay

To assess metalloproteinase activity in MDA-MB-231 cell culture supernatants, a gelatin-zymography was performed following standard procedures modified by Lima et al [12]. Cell culture supernatants were mixed with 5x non-reducing buffer and separated in SDS–

polyacrylamide gels (10% w/v acrylamide) copolymerized with 1% (w/v) gelatin. After electrophoresis, the gel was washed twice for 30 minutes in washing buffer, followed by a 5–10 minute rinse in incubation buffer containing Triton X-100. The buffer was then replaced with fresh incubation buffer, and the gel was incubated at 37°C for 24 hours. To visualize gelatinase activity, the gel was stained with Coomassie blue for 30 minutes and rinsed with distilled water. White bands indicated areas of gelatinase activity, and protein band intensities were determined using a scanner.

Immunoblot Analysis

Immunoblot analysis was conducted to evaluate the expression levels of epithelial-mesenchymal transition (EMT)-related proteins, including an epithelial marker (E-cadherin), a mesenchymal marker (vimentin), and upstream regulatory proteins involved in the migration pathway (NF-κBp65 and p-NF-κBp65). These protein levels were assessed in MDA-MB 231 cells following exposure to various concentrations of AL crud extract for 12 and 24 hours. Cells were first washed with cold 1× PBS and lysed with RIPA buffer at 4°C. The lysates were then centrifugated at 13,000 rpm for 20 minutes, and the supernatants were collected. Protein samples were mixed with 5× loading buffer and heated at 95°C for 5 minutes before being subjected to electrophoresis and transferred onto a polyvinylidene difluoride (PVDF) membrane. To block non-specific binding, the PVDF membranes was incubated with 5% skimmed milk dissolved in PBS for 1 hour. It was then incubated overnight at 4°C with primary antibodies diluted in 2.5% skimmed milk in PBS containing 0.1% Tween-20. The primary antibodies used included vimentin (D21H3), E-cadherin (24E10), NF-κBp65 (D14E12), p-NF-κBp65 (Ser536) (93H1), and β-Actin (8H10D10) (Cell Signaling Technology, Danvers, MA, USA). Detection of target proteins was performed using HRP-conjugated secondary antibodies (goat anti-rabbit and/or anti-mouse). Chemiluminescent signals were generated by exposing the PVDF membrane to an Enhanced Chemiluminescence (ECL) substrate or an alternative chemiluminescent reagent. The emitted light signals were captured using an ImageQuant LAS 5000 Chemiluminescent Imaging System (GE, Boston, MA, USA), and protein signal intensities were quantified using ImageJ software.

Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM) from at least three independent experiments. Differences between each experimental group and the control group were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Statistical significance was set at $p < 0.05$. Data analysis was performed using GraphPad Prism 5.0 software (San Diego, CA, USA).

Results

HPLC Chromatographic Profiling of AL Crude Extract

High-Performance Liquid Chromatography (HPLC) was used to analyze the chemical composition of the AL crude extract, aiming to identify its primary bioactive constituents. A chromatogram was generated using an HPLC system, with a reference standard mixture for comparison. This mixture contained two known compounds: beta-eudesmol (BE) at 2.2237 mg/ml, and atractylodin (Atr) at 500 µg/mL (Figure 1). Quantitative analysis of the AL crude extract revealed concentrations of BE and Atr at 0.59 mg/ml (2.95%) and 337.17 µg/mL (13.91%), respectively. These findings confirm that beta-eudesmol and atractylodin are the major bioactive compounds in the extract, both of which have been previously recognized for their anti-cancer properties. Based on these results, triple-negative breast cancer (TNBC) was selected as a model to further investigate the anti-cancer potential of AL.

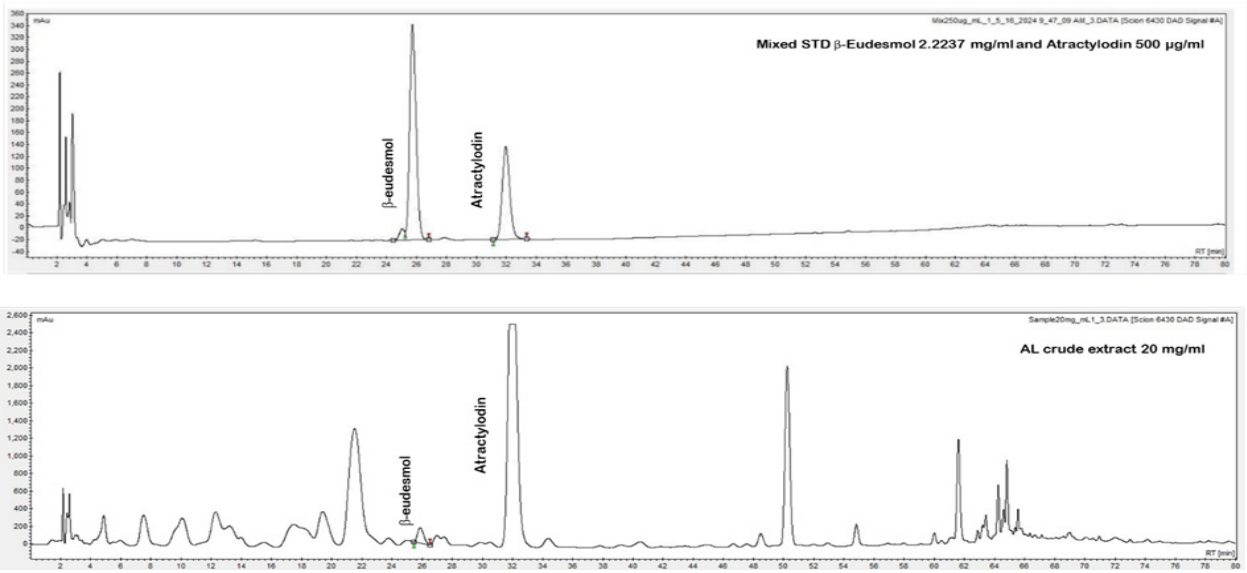

Figure 1. HPLC Chromatogram of the Reference Standard Mixture Containing 2.2237 mg/mL of β-eudesmol and 500 µg/mL of Atractylodin.

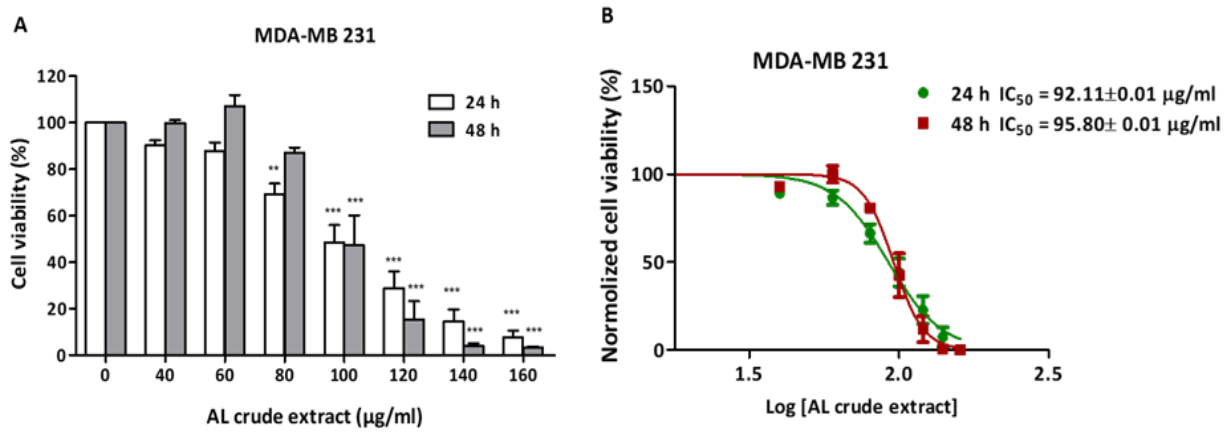

Figure 2. Bar Graphs Depict the Cell Viability (%) of MDA-MB 231 (A) cells after exposure to various concentrations of AL crude extract for 24 and 48 hours. Line graphs illustrate the half-maximal inhibitory concentration (IC50). Cell viability was assessed using the MTT assay. Data are presented as mean±SEM from three independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001 vs untreated control cells.

AL Crude Extract Inhibits BC Cell Proliferation

To evaluate the anti-cancer effects of AL on TNBC, MDA-MB 231 cells were treated with AL crude extract at concentrations ranging from 0 to 160 µg/ml for 24 and 48 hours. Cell viability was then assessed using the MTT assay. As shown in Figure 2A, the half-maximal inhibitory concentrations (IC_{50}) values of AL extract were 92.11±0.01 µg/mL at 24 hours and 95.80±0.01 µg/ml at 48 hours (Figure 2B). These results indicate that AL exerts a dose-dependent anti-proliferative effect on MDA-MB-231 cells. Based on these findings, sub-lethal doses (maintaining over 75% cell viability) were selected for further investigation of the extract's anti-metastatic properties.

AL Crude Extract Inhibits BC Cell Migration

To evaluate the effect of AL crude extract on cell migration, a wound healing assay was performed using sublethal doses determined from the previous MTT assay. A scratch was created in the MDA-MB-231cell monolayer, and images were captured to assess migration. The results demonstrated that AL crude extract inhibited cell migration in a dose-dependent manner (Figure 3A). After 24 hours of treatment with 40, 60, and 80 µg/ml of AL crude extract, the percentage of wound closure was significantly reduced compared to the untreated control. The mean (± SEM) wound closure percentages for MDA-MB 231 cells were 33.54±3.51%, 27.14±3.18%, and 23.62±5.92%, respectively (Figure 3B). These findings suggest that AL effectively suppresses cancer cell migration in a dose-dependent manner. To further investigate its anti-metastatic potential, an assay was conducted to assess the effect of AL on MMP activity.

AL Crude Extract Suppresses MMP-9 Activity in BC Cells

To evaluate the effect of the AL crude extract on MMP-9 and MMP-2 activity, a gelatin zymography assay was performed using MDA-MB 231 cells. The cells were treated with previously determined sublethal concentrations of AL crude extract, and the gelatinolytic activity of MMP-9 and MMP-2 in the extracellular media was analyzed after 24 hours (Figure 4A). The results

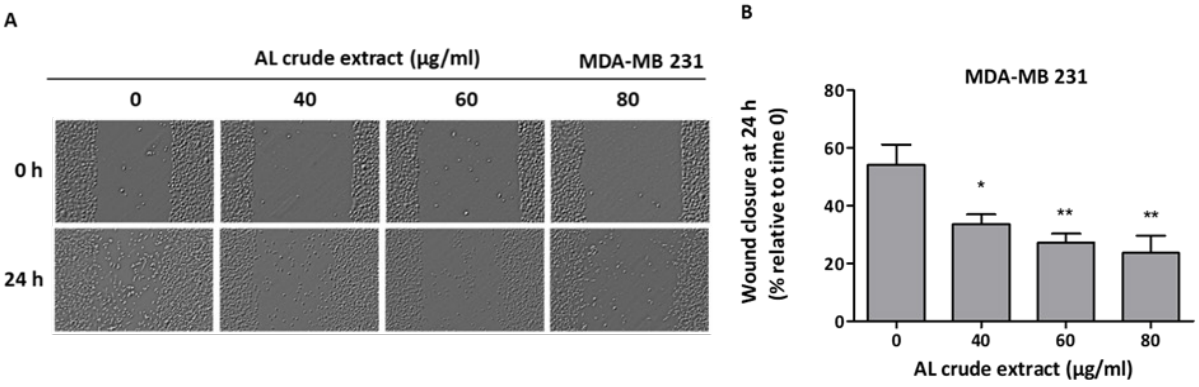

Figure 3. AL Crude Extract Inhibited Wound Closure in MDA-MB 231 Cells. (A) Representative images show cell migration following exposure to 0, 40, 60, and 80 µg/ mL of AL crude extract. Images were captured at 0 and 24 hours to assess wound closure. (B) Bar graphs quantify cell migration as the percentage of wound closure at 24 hours. Data are presented as mean ± SEM from three independent experiments. **p < 0.01, ***p < 0.001 vs untreated control cells.

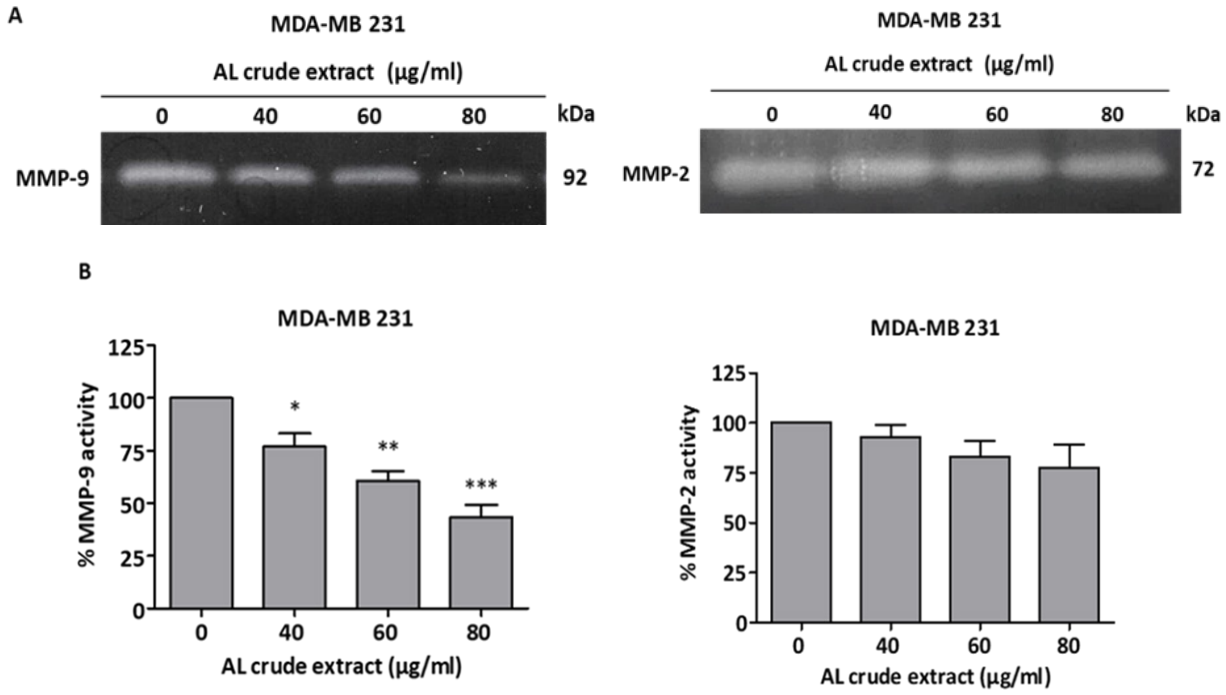

Figure 4. Gelatinolytic Activity by AL Crude Extract in MDA-MB 231 Cells. (A) Zymographic profiles of MMP-9 and MMP-2 activities following exposure to 0, 40, 60, and 80 μg/mL of AL crude extract. Clear bands against a dark blue background indicated gelatinase activity. (B) Bar graphs quantify the percentage of MMP-9 and MMP-2 activities after 24 hours of exposure. Data are presented as mean ± SEM from three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 vs untreated control cells.

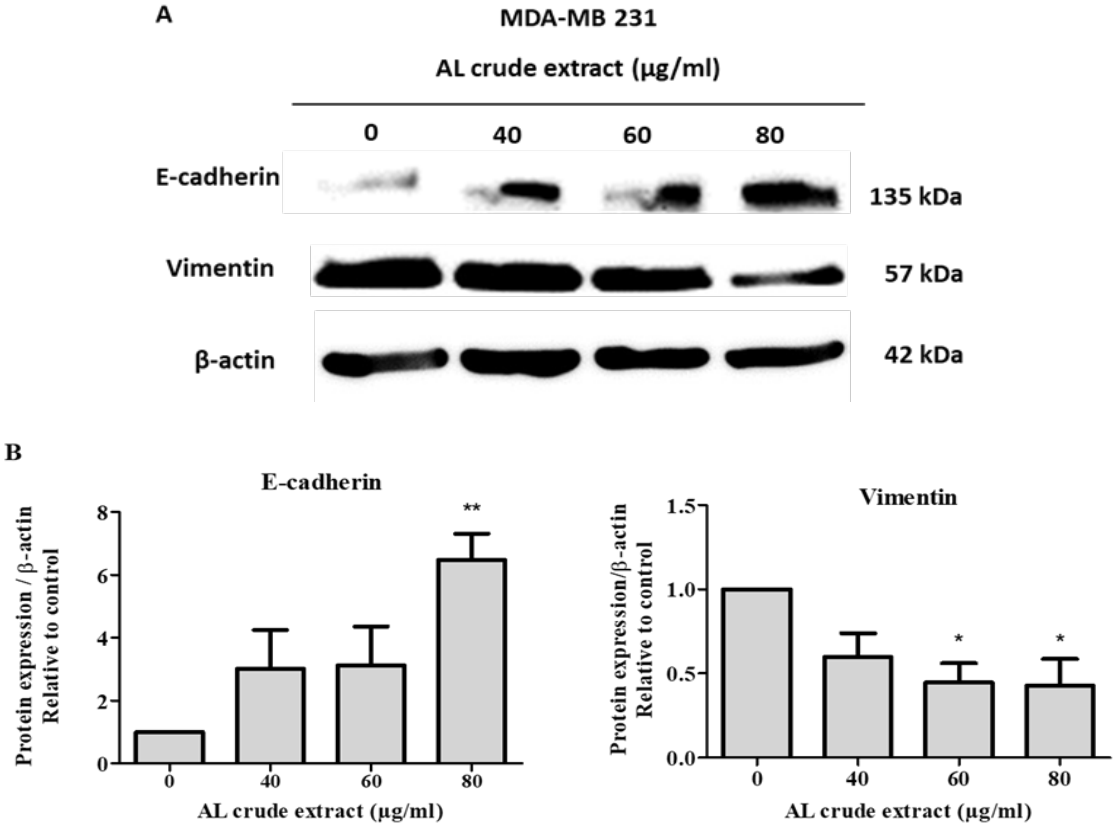

Figure 5. Immunoblot Analysis of the Effect of AL Crude Extract on EMT Marker Expression in MDA-MB-231 Cells. (A) Representative protein bands showing the expression levels of E-cadherin and vimentin in cells treated with 0, 40, 60, and 80 μg/mL of AL crude extract for 24 hours. (B) Bar graphs display normalized E-cadherin and vimentin expression relative to β-actin, which served as the internal loading control. Data are presented as means ± SEM from three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 vs. untreated control cells (one-way ANOVA).

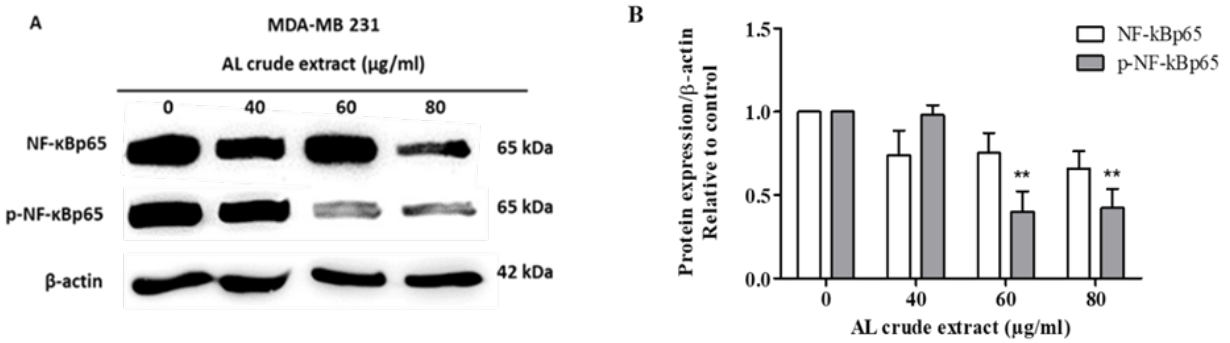

Figure 6. Immunoblot Analysis of the Effect of AL Crude Extract on NF-κBp65 and p-NF-κBp65 Expression in MDA-MB-231 Cells. (A) Representative protein bands showing NF-κBp65 and p-NF-κBp65 expression levels in cells treated with 0, 40, 60, and 80 µg/ mL of AL crude extract for 12 hours. (B) Bar graphs display normalized NF-κBp65 and p-NF-κBp65 expression relative to β-actin. β-actin served as the internal loading control. Data are presented as means ± SEM from three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 vs. untreated control cells (one-way ANOVA).

showed that AL crude extract significantly inhibited MMP-9 activity in a dose-dependent manner. Specifically, the mean (± SEM) MMP-9 activity in MDA-MB-231 cells treated with 40, 60, and 80 µg/mL of AL crude extract was reduced to 76.92±6.40%, 60.62±4.76%, and 43.25±6.01%, respectively. In contrast, MMP-2 activity remained largely unaffected (Figure 4B). These findings indicate that AL crude extract effectively suppresses MMP-9-mediated gelatin degradation, which may contribute to its anti-metastatic potential. To further investigate its mechanism of action, key signaling molecules involved in migration and invasion were selected for analysis.

AL Crude Extract Suppresses EMT-Related Proteins via NF- kB Signaling

The epithelial-to-mesenchymal transition (EMT) is a key process in cancer progression and metastasis, characterized by decreased E-cadherin and increased vimentin expression. In MDA-MB-231 cells, the control group exhibited low E-cadherin levels and high vimentin levels, indicating the loss of epithelial characteristics and the occurrence of EMT. However, treatment with 80 µg/mL of AL crude extract significantly increased E-cadherin expression, while vimentin expression was significantly reduced at 60, and 80 µg/ml (Figure 5A and B). The NF-kB signaling pathway is known to regulate cancer cell invasiveness and migration. To assess its involvement, the expression levels of NF-κBp65 and its phosphorylated form (p-NF-κBp65 at Ser536) were analyzed. After 12 hours of exposure to AL crude extract, total NF-κBp65 levels showed a decreasing trend, though the reduction was not statistically significant. In contrast, the phosphorylated form (p-NF-κBp65 ser536) was significantly reduced at 60 and 80 µg/mL of AL crude extract (Figure 6A and B). These findings suggest that NF-kB p65 phosphorylation plays a role in MDA-MB-231 cell metastasis. Furthermore, AL crude extract reduces cell migration and suppresses MMP-9 degradation activity after 24 hours of treatment, indicating its potential anti-metastatic effects.

Discussion

Breast cancer is the leading cause in cancer-related deaths among women worldwide. One of the major challenges in breast cancer treatment is the limited availability of effective medications, which is further exacerbated by the rapid emergence of drugs resistance, particularly in triple-negative breast cancer (TNBC) [13]. The primary cause for mortality in breast cancer patients is the metastasis to vital organs such as the liver, lungs, and brain, which can occur even in the early stages of disease progression. As a result, standard treatments often yield suboptimal outcomes, including drug resistance, cancer recurrence, and severe adverse effects [14].

Given these challenges, there is a growing need to explore complementary and alternative therapeutic strategies. Previous studies have reported that AL crude extract possesses anti-cancer properties [7]. This study investigated its cytotoxic effects on breast cancer cells, revealing a dose- and time-demependent cytotoxicity, consistent prior findings demonstrating similar effects in human gastric cancer cells [15]. Additionally, an ethanolic extract from AL rhizomes was shown to inhibit cholangiocarcinoma (CCA) with an IC_{50} of 24.09±3.40 µg/mL (mean±SD) [16]. Moreover, sesquiterpenoids from Atractylodes ovata have been reported to exhibit cytotoxic activity against leukemia cells [17]. Since previous phytochemical analyses have confirmed the presence of sesquiterpenoids in AL [10], our findings suggest that AL crude extract may have broad anti-cancer potential by reducing cell viability in multiple cancer types.

A key factor contributing to the high mortality rate of aggressive breast cancer is its strong metastatic potential. Early detection significantly improves survival rates; however, breast cancer is often diagnosed only after it has metastasized [1]. TNBC is known to exhibit high levels of vimentin, N-cadherin, and cadherin-11 markers indicative of epithelial-to-mesenchymal transition (EMT), a process that facilitates metastasis through extracellular matrix (ECM) remodeling via matrix metalloproteinases (MMPs) [18]. As expected, E-cadherin expression is typically absent in TNBC [19]. In this study, treatment

with AL crude extract suppressed breast cancer cell migration, upregulated the epithelial marker E-cadherin in MDA-MB-231 cells, and downregulated the mesenchymal marker vimentin, suggesting an EMT-inhibitory effect.

EMT is a complex process regulated by multiple signaling pathways [20]. Among these, the NF-kB pathway plays a crucial role in stabilizing key EMT regulators such as Snail and Twist1, which drive the transition to a more motile mesenchymal state [21-23]. Inhibition of NF-kB signaling has been shown to reduce cancer cell migration and invasion. To investigate the involvement of this pathway, we analyzed the expression of NF-κBp65 and its phosphorylated form at Serine 536, which is associated with enhanced migration capability in breast cancer cells. Our findings demonstrated that AL crude extract significantly reduced p-NF-κBp65 levels in MDA-MB-231 cells (Figure 6), supporting the hypothesis that AL suppresses cancer cell migration by modulating NF-kB signaling.

Furthermore, previous studies have established that MMP-2 and MMP-9 play crucial roles in ECM degradation, promoting tumor invasion and metastasis [24]. MMP-2 and MMP-9 expressions are significantly higher in breast cancer cells (MDA-MB 231 and MCF-7) compared to normal breast cells (eHS578Bst) [14]. Our gelatin zymography results suggest that AL crude extract inhibits MMP-9 activity, likely through NF-kB suppression in aggressive breast cancer. These findings are consistent with prior research showing that andrographolide, a diterpenoid lactone from Andrographis paniculata, suppresses MDA-MB-231 migration and invasion by inhibiting NF-kB-dependent MMP-9 production [25]. Similarly, Kang et al. reported that Glaucine, an alkaloid isolated from Corydalis turtschaninovii tubers, inhibits MDA-MB-231 invasion by suppressing MMP-9 via the NF-kB pathway [26]. These studies highlight the critical role of NF-kB in breast cancer metastasis and further support AL crude extract as a promising anti-metastatic agent. However, the major bioactive compounds, such as atractylodin and ß-eudesmol, should be tested to clarify the synergistic effects in TNBC.

Our findings provide additional evidence that AL crude extract inhibits breast cancer cell proliferation and migration. The observed suppression of MMP-9 activity and EMT-related markers is likely mediated through NF-kB signaling inhibition. However, cytotoxic insight, including apoptosis and cell cycle analysis, will be needed to further explore. Future studies should focus on identifying the major bioactive compounds within AL crude extract to enhance therapeutic precision and reliability. With an understanding of the molecular mechanisms underlying its anti-cancer properties, AL crude extract may serve as a valuable adjunct to conventional chemotherapy, offering novel treatment strategies based on natural compounds.

Author Contribution Statement

Khunchai, Sasiprapa; Panya, Aussara; Pongcharoen, Sutatip; Horpaopan, Sukanya; Yenchitsomanus, Pa-thai; Sudsaward, Sangkab conceptualized the research project, designed the overall experimental studies, and edited manuscript. Changtong, Akawan; Sereesantiwong, pattareeya; Wongho, Warintorn conducted the experiments and collected the data. Changtong, Akawan; Khunchai, Sasiprapa; Sudsaward, Sangkab analyzed the data, interpreted the results, and drafted the manuscript.

Acknowledgements

General

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Scientific Approval

This research has been approved in accordance with the announcement from the Graduate School at Naresuan University, Thailand.

Conflict of Interest

The authors declare no potential conflict of interest.

Ethical Declaration

This work has been approved by the Scientific Committee of the Institutional Review Board at Naresuan University (COE No. 019/2023).

References

1. Park M, Kim D, Ko S, Kim A, Mo K, Yoon H. Breast Cancer Metastasis: Mechanisms and Therapeutic Implications. Int J Mol Sci. 2022;23(12):6806. https://doi.org/10.3390/ijms23126806
2. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-Negative Breast Cancer: Clinical Features and Patterns of Recurrence. Clin Cancer Res. 2007;13(15):4429-34. https://doi.org/10.1158/1078-0432.CCR-06-3045
3. André F, Zielinski CC. Optimal strategies for the treatment of metastatic triple-negative breast cancer with currently approved agents. Ann Oncol. 2012;23:vi46-vi51. https://doi.org/10.1093/annonc/mds195
4. Acharya B, Chajaroenkul W, Na-Bangchang K. beta-Eudesmol Inhibits the Migration of Cholangiocarcinoma Cells by Suppressing Epithelial-Mesenchymal Transition via PI3K/AKT and p38MAPK Modulation. Asian Pac J Cancer Prev. 2022;23(8):2573-81. https://doi.org/10.31557/APJCP.2022.23.8.2573
5. Liu S, Li L, Ren D. Anti-Cancer Potential of Phytochemicals: The Regulation of the Epithelial-Mesenchymal Transition. Molecules. 2023;28(13):5069. https://doi.org/10.3390/

molecules28135069
6. Felipe Lima J, Nofech-Mozes S, Bayani J, Bartlett JM. EMT in Breast Carcinoma-A Review. J Clin Med. 2016;5(7):65. https://doi.org/10.3390/jcm5070065
7. Koonrungsesomboon N, Na-Bangchang K, Karbwang J. Therapeutic potential and pharmacological activities of *Atractylodes lancea* (Thunb.) DC. Asian Pac J Trop Med. 2014;7(6):421-8. https://doi.org/10.1016/S1995-7645(14)60069-9
8. Li Z, Song Y, Hou W, Qi Y, Lu X, Xue Y, et al. Atractylodin induces oxidative stress-mediated apoptosis and autophagy in human breast cancer MCF-7 cells through inhibition of the P13K/Akt/mTOR pathway. J Biochem Mol Toxicol. 2022;36(8):e23081. https://doi.org/10.1002/jbt.23081.
9. Li Z, Li J, Liu X, Liu Y, Chen H, Sun X. beta-eudesmol inhibits cell proliferation and induces ferroptosis via regulating MAPK signaling pathway in breast cancer. Toxicon. 2024;237:107529. https://doi.org/10.1016/j.toxicon.2023.107529
10. Na-Bangchang K, Plengsuriyakarn T, Karbwang J. Research and Development of *Atractylodes lancea* (Thunb) DC. as a Promising Candidate for Cholangiocarcinoma Chemotherapeutics. Evid Based Complement Alternat Med. 2017;2017:5929234. https://doi.org/10.1155/2017/5929234
11. Sun J, Luo H, Jiang Y, Wang L, Xiao C, Weng L. Influence of Nutrient (NPK) Factors on Growth, and Pharmacodynamic Component Biosynthesis of Atractylodes chinensis: An Insight on Acetyl-CoA Carboxylase (ACC), 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGR), and Farnesyl Pyrophosphate Synthase (FPPS) Signaling Responses. Front Plant Sci. 2022;13:799201. https://doi.org/10.3389/fpls.2022.799201
12. Lima AI, Mota J, Monteiro SA, Ferreira RM. Legume seeds and colorectal cancer revisited: Protease inhibitors reduce MMP-9 activity and colon cancer cell migration. Food Chem. 2016;197(Pt A):30-8. https://doi.org/10.1016/j.foodchem.2015.10.063
13. Heers H, Stanislaw J, Harrelson J, Lee MW. Valproic acid as an adjunctive therapeutic agent for the treatment of breast cancer. Eur J Pharmacol. 2018;835:61-74. https://doi.org/10.1016/j.ejphar.2018.07.057
14. Li H, Qiu Z, Li F, Wang C. The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. Oncol Lett. 2017;14(5):5865-70. https://doi.org/10.3892/ol.2017.6924
15. Zhao M, Wang Q, Ouyang Z, Han B, Wang W, Wei Y, et al. Selective fraction of *Atractylodes lancea* (Thunb.) DC. and its growth inhibitory effect on human gastric cancer cells. Cytotechnology. 2014;66(2):201-8. https://doi.org/10.1007/s10616-013-9559-1
16. Mahavorasirikul W, Viyanant V, Chaijaroenkul W, Itharat A, Na-Bangchang K. Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro. BMC Complement Altern Med. 2010;10:55. https://doi.org/10.1186/1472-6882-10-55
17. Wang CC, Chen LG, Yang LL. Cytotoxic activity of sesquiterpenoids from Atractylodes ovata on leukemia cell lines. Planta Med. 2002;68(3):204-8. https://doi.org/10.1055/s-2002-23144
18. Micalizzi DS, Ford HL. Epithelial-mesenchymal transition in development and cancer. Future Oncol. 2009;5(8):1129-43. https://doi.org/10.2217/fon.09.94
19. Zhang YQ, Wei XL, Liang YK, Chen WL, Zhang F, Bai JW, et al. Over-Expressed Twist Associates with Markers of Epithelial Mesenchymal Transition and Predicts Poor Prognosis in Breast Cancers via ERK and Akt Activation. PLoS One. 2015;10(8):e0135851. https://doi.org/10.1371/journal.pone.0135851
20. Xu W, Yang Z, Lu N. A new role for the PI3K/Akt signaling pathway in the epithelial-mesenchymal transition. Cell Adh Migr. 2015;9(4):317-24. https://doi.org/10.1080/19336918.2015.1016686
21. Li CW, Xia W, Huo L, Lim SO, Wu Y, Hsu JL, et al. Epithelial-mesenchymal transition induced by TNF-α requires NF-kB-mediated transcriptional upregulation of Twist1. Cancer Res. 2012;72(5):1290-300. https://doi.org/10.1158/0008-5472.CAN-11-3123
22. Li S, Kendall SE, Raices R, Finlay J, Covarrubias M, Liu Z, et al. TWIST1 associates with NF-kB subunit RELA via carboxyl-terminal WR domain to promote cell autonomous invasion through IL8 production. BMC Biol. 2012;10:73. https://doi.org/10.1186/1741-7007-10-73
23. Wu Y, Deng J, Rychahou PG, Qiu S, Evers BM, Zhou BP. Stabilization of snail by NF-kappaB is required for inflammation-induced cell migration and invasion. Cancer Cell. 2009;15(5):416-28. https://doi.org/10.1016/j.ccr.2009.03.016
24. Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J. 2011;278(1):16-27. https://doi.org/10.1111/j.1742-4658.2010.07919.x
25. Zhai Z, Qu X, Li H, Ouyang Z, Yan W, Liu G, et al. Inhibition of MDA-MB-231 breast cancer cell migration and invasion activity by andrographolide via suppression of nuclear factor-κB-dependent matrix metalloproteinase-9 expression. Mol Med Rep. 2015;11(2):1139-45. https://doi.org/10.3892/mmr.2014.2872
26. Kang H, Jang SW, Pak JH, Shim S. Glaucine inhibits breast cancer cell migration and invasion by inhibiting MMP-9 gene expression through the suppression of NF-kB activation. Mol Cell Biochem. 2015;403(1-2):85-94. https://doi.org/10.1007/s11010-015-2339-9.