

## Anti-migration and Anti-invasion Effects of *Mitragyna Speciosa* Polar Extract on A549 Cell

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### Abstract

*Mitragyna speciosa* (commonly known as Kratom) is a tropical plant native to Southeast Asia and a member of the Rubiaceae family. This study aimed to investigate the anticancer potential of crude *M. speciosa* leaf extracts against non-small cell lung cancer (NSCLC) cells. Dried leaves were extracted using 95% ethanol or 95% methanol at a 1:10 w/v ratio via maceration. The cytotoxic effects of the extracts were evaluated using the MTT assay, and results were expressed as IC<sub>50</sub> values. Cell migration and invasion were assessed using wound healing and Transwell invasion assays, respectively. All experiments were conducted in triplicate. The MTT assay revealed that both ethanolic and methanolic extracts significantly reduced the viability of A549 cells in a dose-dependent manner. The methanolic extract exhibited greater potency, with an IC<sub>50</sub> of 283 µg/mL, compared to 295 µg/mL for the ethanolic extract. In the wound healing assay, both extracts inhibited A549 cell migration, with the methanolic extract showing stronger suppression at concentrations of 50, 100, and 250 µg/mL. The ethanolic extract also reduced migration but required higher concentrations to achieve similar effects. Transwell invasion assay results further demonstrated that both extracts suppressed cell invasion, with the methanolic extract being more effective at lower concentrations (50 and 100 µg/mL). Furthermore, real-time RT-PCR analysis revealed that the extracts upregulated E-cadherin and, in the methanolic group, downregulated MMP-2 expression, suggesting suppression of epithelial-mesenchymal transition (EMT)-associated invasion. These findings highlight the potential of *M. speciosa* as a source of bioactive compounds with anti-migratory and anti-invasive properties. Further investigation is warranted to elucidate the underlying mechanisms and evaluate its potential for development as a natural anticancer agent.

**Keywords:** Non-small cell lung cancer, *Mitragyna speciosa*, MTT assay, Wound healing, Transwell invasion assay

### Introduction

Cancer is one of the world's main health problems. In Thailand, cancer is one of the leading causes of death from disease. One of the main incidences of cancers in the Thai population is lung cancer [1]. The incidence of lung cancer among males is 22.7 per 100,000 population. It is the 2<sup>nd</sup> most common cancer, after liver cancer. Among females, the incidence is 10.1 per 100,000 population and is the 4<sup>th</sup> most common after breast cancer, cervical cancer, and liver cancer [2]. Lung cancer is caused by abnormalities of cells within the body that are affected by the behavior of a person's lifestyle, such as smoking cigarettes and working with

asbestos (e.g., mining). The mutated cells have a high proliferation rate and can spread to other organs. Lung cancer is classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with the latter accounting for approximately 85% of all cases. NSCLC is characterized by slow growth but a high potential for local invasion and distant metastasis, which contributes significantly to its poor prognosis and high mortality.

Currently, non-small cell lung cancer (NSCLC) is treated using a combination of therapeutic modalities depending on the stage and molecular profile of the tumor. These include surgery for localized tumors,

chemotherapy and radiotherapy for systemic control, as well as targeted therapy and immunotherapy for advanced-stage disease [3]. Although these treatments have improved overall survival in many patients, several challenges remain. Chemotherapy and radiotherapy are associated with non-specific cytotoxicity, resulting in adverse effects such as fatigue, nausea, immunosuppression, and organ toxicity [4]. In addition, patients may develop resistance to targeted agents or immunotherapies over time, limiting long-term effectiveness. The high cost and limited availability of certain therapies also restrict access, particularly in low-resource settings [5]. These limitations underscore the need for alternative or complementary treatment approaches, such as plant-based therapies, which may offer multi-targeted action with lower toxicity profiles. Nowadays, alternative therapies, such as using plants or herbal extracts, are widely studied, which may be effective and reduce the side effects of treatment. For example, Juthathip and colleagues suggested that 4 Thai herbs, including ethyl acetate extracts of *Bridelia ovata*, *Croton oblongifolius*, and *Erythrophleum succirubrum*, and an ethanolic extract of *Erythrophleum succirubrum*, possessed anticancer effects on lung cancer [6].

*M. speciosa* is a tropical plant usually found in Southeast Asia, locally known as Kratom in Thailand. It is a member of the Rubiaceae family. General studies reported that *M. speciosa* was used as a stimulant by workers and for pain relief [7]. Kratom was classified as a narcotic in Thailand until now it is legalized since August 2021. Among its major alkaloids, mitragynine and 7-hydroxymitragynine are the most abundant and pharmacologically active constituents. These compounds have been reported to exert antioxidant, anti-inflammatory, and anticancer effects through induction of apoptosis and suppression of cell proliferation in various cancer models [8-11]. A previous study investigated mitragynine, an indole alkaloid in *M. speciosa*, combined with cisplatin to treat nasopharyngeal carcinoma. Gregory and colleagues reported that mitragynine could be a potential chemosensitizer for cisplatin [8]. Moreover, Goh's research found that mitragynine exhibits potent and selective cytotoxic effects via apoptotic pathway and anti-proliferation efficacy against K562 and HCCT 116 cancer cell lines [9]. Interestingly, it may affect cancer cells. Consequently, this study aims to investigate the

effects of *M. speciosa* extract on the proliferation, migration, and invasion of non-small cell lung cancer (NSCLC) cells. Understanding these effects may provide insights into the potential of *M. speciosa* as an alternative or complementary therapeutic approach for lung cancer treatment.

## Materials and methods

### Plant materials and extraction

Dried leaves of *M. speciosa* were extracted using 2 solvents: 95% ethanol (ACI Labscan) or 95% methanol (ACI Labscan). The maceration process was conducted at a ratio of 1:10 w/v (weight to volume). The leaves were macerated in the solvent for 7 days at room temperature. After maceration, the mixture was filtered using filter paper (Whatman) and a funnel to remove solid residues. The filtrate was then evaporated using a rotary evaporator to obtain the concentrated extract. The dried extract samples were collected and stored at room temperature for further experiments. The percentage yield of the extract was calculated using the formula:

$$\% \text{ yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100\%.$$

### Cell culture

The human non-small cell lung cancer cell line (NSCLC) was used in this study that the A549 (ATCC CCL-185) was kindly received from Dr. Santi Phosri. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. They were maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub> until they reached the appropriate confluence.

### MTT assay

The MTT assay was performed to evaluate the cytotoxic effects of the *M. speciosa* extracts on A549 cells. Cells were seeded in a 96-well plate at a density of 3,000 cells per well and allowed to attach overnight. Following attachment, the cells were treated with varying concentrations of ethanolic and methanolic extracts (10, 25, 50, 100, 250, 500, and 1,000 µg/mL) for 24 h. After the treatment period, MTT solution (20 µL) was added to each well and incubated for 4 h at 37 °C. The medium was then removed, and 100 µL of

DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader.

### Wound-healing assay

Wound-healing assay was conducted to assess effects of the extracts on cell migration. A549 cells were seeded in 24-well plates (Corning Costar) and grown to 90% confluence. A scratch was created in the monolayer using a 200- $\mu$ L pipette tip, and the wells were washed with PBS to remove detached cells and debris. The cells were then treated with varying concentrations of extracts (1, 10, and 50  $\mu$ g/mL). Images of the wound were captured at 0, 6, 12, 18, and 24 h using a phase-contrast microscope. The wound closure was quantified by measuring the remaining wound area at each time point. The wound-healing assay procedure was adapted with minor modifications from Rodriguez *et al.* [12].

### Transwell invasion assay

Transwell invasion assay was used to evaluate inhibitory effects of the extracts on cancer cell invasion. Transwell inserts were coated with Matrigel to simulate the extracellular matrix. The treated A549 cells (with 50, 100, and 250  $\mu$ g/mL of extracts) were seeded in the upper chamber, while the lower chamber contained culture medium. After 24 h, non-invasive cells were removed from the upper chamber, and invaded cells on the lower surface of the membrane were fixed with methanol, stained with crystal violet, and visualized under a stereoscope. The assay procedure was performed with minor modifications from Justus *et al.* [13].

### Real time RT-PCR

A549 cells were treated with ethanolic or methanolic *M. speciosa* extracts at a concentration of

500  $\mu$ g/mL for 12 h, then harvested. Total RNA was isolated from A549 cells with Trizol® reagent (Invitrogen, CA, US). Two hundred ng of RNA was allowed both reverse transcription and real time-PCR using QuantiNova® SYBR® Green RT-PCR kit (Qiagen, Germany) following the manufacturer's instruction. The primers that were used are e-cadherin: (forward) GAACAGCACGTACACAGCCCT, (reverse) GCAGAAGTGTCCCTGTTCCAG; ICAM-1: (forward) GGCTGGAGCTGTTTGAGAAC, (reverse) ACTGTGGGGTTCAACCTCTG; MMP-2: (forward) GGTTTCATTTGGCGGACTG, (reverse) AGGCTGGTCAGTGGCTTG; and GAPDH: (forward) GAAGGTGAAGGTCCGAGTCA (reverse) TTGAGGTCAATGAAGGGGTC. The relative mRNA expression levels were normalized with endogenous control GAPDH gene expression. The values were expressed as fold change ( $2^{-\Delta\Delta Ct}$ ), where  $\Delta\Delta Ct = (\Delta Ct_{Test} / \Delta Ct_{Control})$ .

### Statistical analysis

All experiments were conducted in triplicate ( $n = 3$ ) and data were presented as mean  $\pm$  standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 8.0.2 (GraphPad Software Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine statistical significance among groups. Differences were considered statistically significant at  $p < 0.05$ .

## Results and discussion

### Extraction yield

The extraction yield of *M. speciosa* leaves using 95% ethanol or methanol was determined. The ethanol extract yielded is 4.44% and the methanol extract yielded is 3.08%.

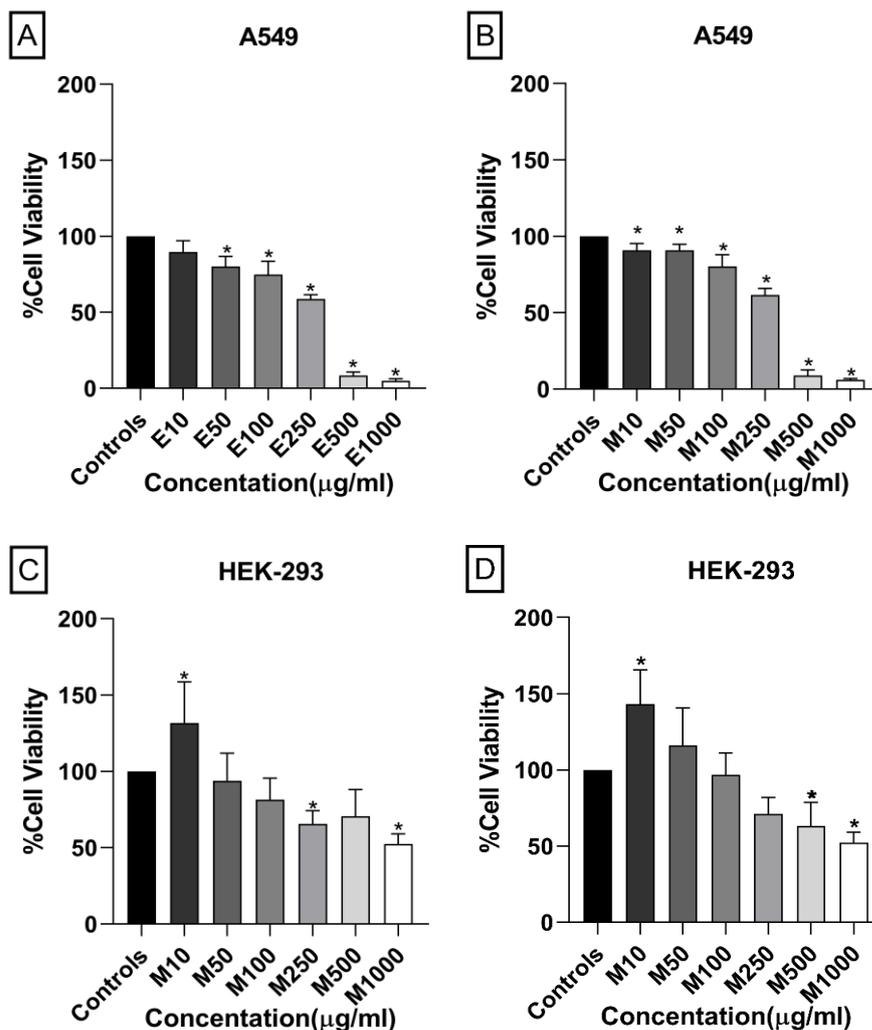
**Table 1** Comparative extraction yield (%) of *M. speciosa* using 95% methanol and 95% ethanol.

Solvent	%Yield
95% Ethanol	4.44
95% Methanol	3.08

### Cytotoxicity of the *M. speciosa* extract by MTT assay

This study evaluated the cytotoxicity of *M. speciosa* extracts using the MTT assay. The A549 cells were exposed to varying concentrations of both ethanolic and methanolic extracts. The results demonstrated that both extracts significantly reduced cell viability in a dose-dependent manner ( $p < 0.05$ ). In the untreated control cells, cell viability was set at 100%. For the ethanolic extract, cell viability decreased to 89.54% at 10  $\mu\text{g/mL}$ , 79.92% at 50  $\mu\text{g/mL}$  and 74.59% at 100  $\mu\text{g/mL}$ . At the higher concentrations, cytotoxicity increased markedly, with cell viability dropping to 58.67% at 250  $\mu\text{g/mL}$ , 8.51% at 500  $\mu\text{g/mL}$ , and only 4.88% at 1000  $\mu\text{g/mL}$  (**Figure 1(A)**). The methanolic extract showed a similar pattern. Cell viability remained high at 90.69 and 90.66% for 10 and 50  $\mu\text{g/mL}$ , respectively. However, it decreased to 78.28% at 100  $\mu\text{g/mL}$ , 61.53% at 250  $\mu\text{g/mL}$  and dropped sharply to 8.80% at 500  $\mu\text{g/mL}$  and 6.01% at 1000  $\mu\text{g/mL}$  (**Figure 1(B)**). The  $\text{IC}_{50}$  values were calculated to be 295  $\mu\text{g/mL}$  for the ethanolic extract and 283  $\mu\text{g/mL}$  for the methanolic extract, indicating potent cytotoxicity against A549 cells, with the methanolic extract showing slightly higher potency. We further investigated the effects on noncancer cell line (non-cancerous HEK-293 cells which kindly received from Assoc. Prof. Dr. Korawinwich Boonpisuttinant), the results showed less toxicity from both extracts (**Figures 1(C)** and **1(D)**). The ethanolic extract maintained 70.53% cell viability at 500  $\mu\text{g/mL}$  and 52.45% at 1000  $\mu\text{g/mL}$ , with an  $\text{IC}_{50}$  exceeding 1000  $\mu\text{g/mL}$ . Similarly, the methanolic extract resulted in 63.3% cell viability at 500  $\mu\text{g/mL}$  and 52.18% at 1000  $\mu\text{g/mL}$  when tested on HEK-293 cells. The results revealed a dose-dependent cytotoxicity for both ethanolic and methanolic extracts, with the

methanolic extract exhibiting slightly higher potency ( $\text{IC}_{50} = 283 \mu\text{g/mL}$ ) compared to the ethanolic extract ( $\text{IC}_{50} = 295 \mu\text{g/mL}$ ). These findings align with previous studies highlighting the anticancer potential of *M. speciosa* alkaloids, suggesting that its alkaloid constituents play a crucial role in its anticancer activity [10]. When comparing these results to prior investigations, *M. speciosa* extracts have been shown to enhance the cytotoxicity of low-dose doxorubicin against A549 cells, indicating potential synergy in combination therapies [14]. Similarly, mitragynine and its analogs exhibit significant cytotoxic effects on various cancer cell lines, reinforcing the selective anticancer activity of *M. speciosa* alkaloids [12]. The observed cytotoxicity in this study could be attributed to the presence of bioactive alkaloids such as mitragynine and speciogynine, which have been reported to induce apoptosis and inhibit cell proliferation [6]. The differential toxicity between cancerous A549 cells and non-cancerous HEK-293 cells further supports the potential therapeutic window of *M. speciosa*. While both extracts exhibited significant cytotoxicity against A549 cells, they demonstrated lower toxicity towards HEK-293 cells, with  $\text{IC}_{50}$  values exceeding 1000  $\mu\text{g/mL}$ . This variability in toxicity across different cell types highlights the need for further *in vivo* validation to ensure selective anticancer effects while minimizing harm to normal cells [15]. Mechanistically, interactions between *M. speciosa* alkaloids and chemotherapeutic agents inhibit cancer cell proliferation and migration, which may partly explain the potent cytotoxic effects observed in this study [16]. Additionally, mitragynine has been reported to modulate cancer cell viability and apoptosis pathways, further supporting its role in cytotoxic activity through apoptosis induction and cell cycle arrest [17].



**Figure 1** Effects of ethanolic and methanolic extracts of *M. speciosa* on cell viability of A549 and HEK-293 Cells: (A) A549 cell treated with the ethanolic extract, (B) A549 cell treated with the methanolic extract, (C) HEK-293 cell treated with the ethanolic extract, (D) HEK-293 cell treated with the methanolic extract. Data are presented as mean  $\pm$  SD from 3 independent experiments ( $n = 3$ ) \* $p < 0.05$  vs controls group.

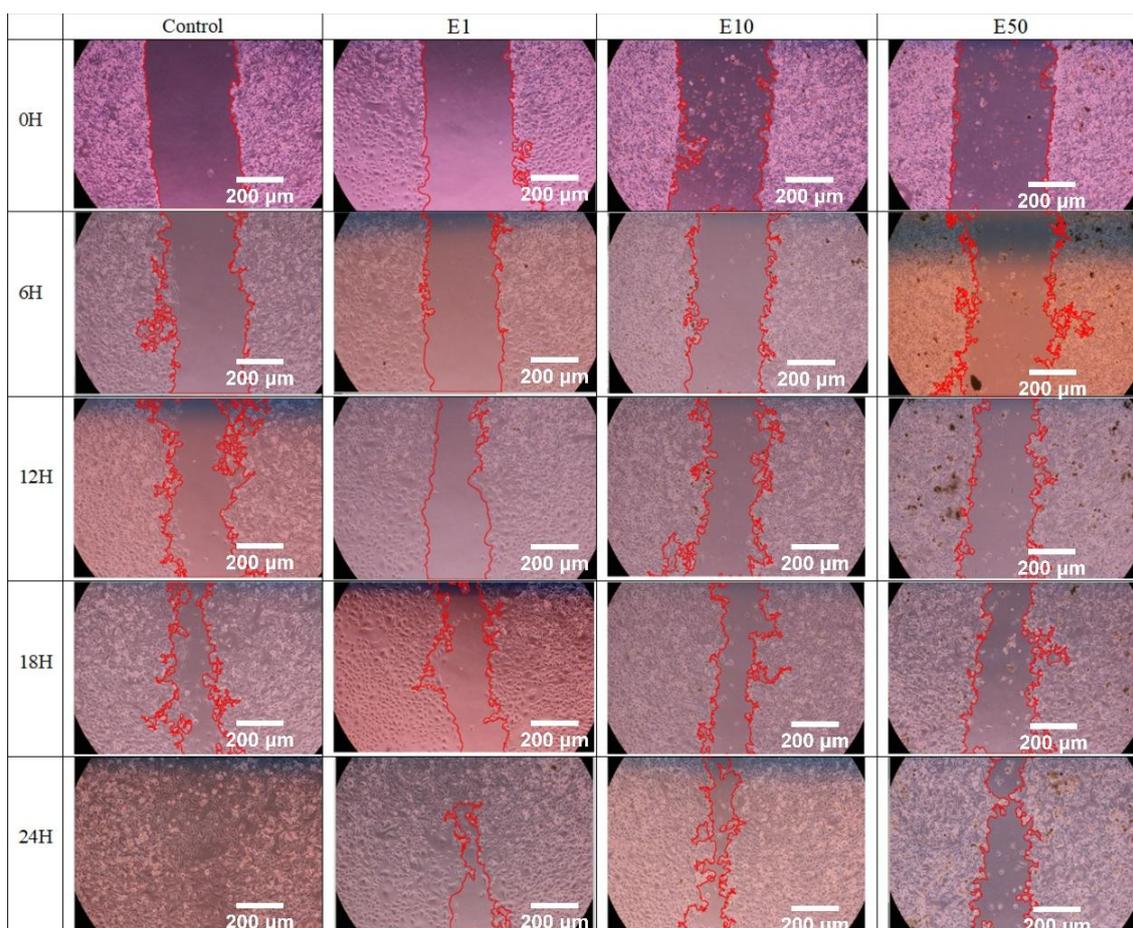
### Wound healing

The wound-healing assay was conducted to evaluate anti-migratory effects of *M. speciosa* extracts on A549 non-small cell lung cancer (NSCLC) cells. Both ethanolic and methanolic extracts were tested at concentrations of 1, 10, and 50  $\mu\text{g/mL}$  with wound closure measured at 0, 6, 12, 18, and 24 h. In the control group, wound healing progressed naturally, reaching 98.64% closure by 24 h. The ethanolic extracts demonstrated dose-dependent inhibition of wound healing (Figure 2). At 1  $\mu\text{g/mL}$  (E1), wound closure reached 78.78% by 24 h. The 10  $\mu\text{g/mL}$  (E10) concentration showed slower healing, achieving 71.91% closure, while the highest concentration of 50  $\mu\text{g/mL}$

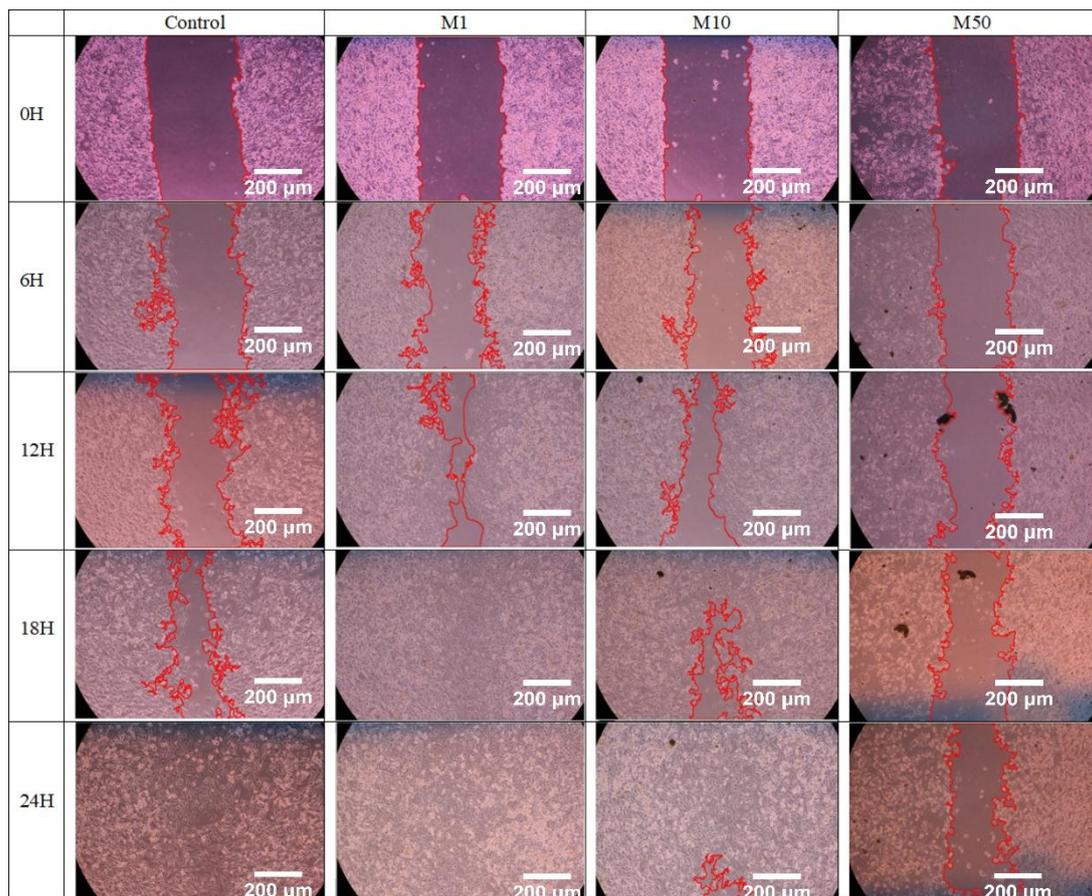
(E50) exhibited the strongest inhibition, with only 67.54% closure by 24 h. Methanolic extracts also displayed concentration-dependent effects (Figure 3). The lowest concentration (1  $\mu\text{g/mL}$ , M1) allowed rapid wound healing, reaching 100% closure by 18 h. At 10  $\mu\text{g/mL}$  (M10), wound closure was reduced to 91.99% by 24 h. The highest concentration (50  $\mu\text{g/mL}$ , M50) showed the most significant inhibition, with only 51.66% closure by 24 h. These results indicate that *M. speciosa* extracts can effectively inhibit NSCLC cell migration in a dose-dependent manner. Higher concentrations of both extract types more potently restricted cell migration, suggesting potential anti-metastatic properties. The methanolic extract at 50

$\mu\text{g/mL}$  demonstrated the strongest inhibitory effect, while lower concentrations, particularly the  $1 \mu\text{g/mL}$  methanolic extract, showed minimal inhibition. Previous research has demonstrated that mitragynine significantly inhibits the migratory capacity of C6 cancer cells at a concentration of  $30 \mu\text{g/mL}$  [16]. Furthermore, when used in combination with cisplatin,

it has been shown to inhibit the migration of nasopharyngeal carcinoma cell lines [6]. Notably, this study revealed that mitragynine, at a concentration of  $50 \mu\text{g/mL}$ , achieved the most effective inhibition of cell migration. Concurrently, it was also found to enhance wound healing in both fibroblast and HUVECs cell lines at lower concentrations [18].



**Figure 1** Wound healing assay showing the closure of the wound area in A549 cells treated with *M. speciosa* extract using ethanol as the solvent at different concentrations ( $1, 10, 50 \mu\text{g/mL}$ ) after 24 h. C = Control, E1 = Ethanolic extract  $1 \mu\text{g/mL}$ , E10 = Ethanolic extract  $10 \mu\text{g/mL}$ , and E50 = Ethanolic extract  $50 \mu\text{g/mL}$ . Images were captured under a phase-contrast microscope at 100 magnification. Scale bar =  $200 \mu\text{m}$ .



**Figure 3** Wound healing assay showing the closure of the wound area in A549 cells treated with *M. speciosa* extract using methanol as the solvent at different concentrations (1, 10, 50  $\mu\text{g/mL}$ ) after 24 h. C = Control, M1 = Methanolic extract 1  $\mu\text{g/mL}$ , M10 = Methanolic extract 10  $\mu\text{g/mL}$ , and M50 = Methanolic extract 50  $\mu\text{g/mL}$ . Images were captured under a phase-contrast microscope at 100 magnification. Scale bar = 200  $\mu\text{m}$ .

### Transwell invasion assay

The effect of *M. speciosa* ethanolic and methanolic extracts on cell invasion was evaluated using the Transwell invasion assay. The ethanolic extract at 50  $\mu\text{g/mL}$  (E50) reduced invasion to 72.39% relative to control, while 100  $\mu\text{g/mL}$  (E100) exhibited a stronger inhibitory effect (60.98%), representing the most effective concentration for the ethanolic extract. At 250  $\mu\text{g/mL}$  (E250), invasion was further suppressed (32.49%), indicating a dose-dependent response (**Figure 4(A)**). Similarly, the methanolic extract at 50  $\mu\text{g/mL}$  (M50) had a minimal impact on invasion (98.33% relative to control), indicating that this concentration does not alter cell migration. However, at 100  $\mu\text{g/mL}$  (M100), the invasion rate was markedly reduced (24.03%), demonstrating the most effective inhibition. Increasing the concentration to 250  $\mu\text{g/mL}$  (M250) further decreased invasion (20.16%),

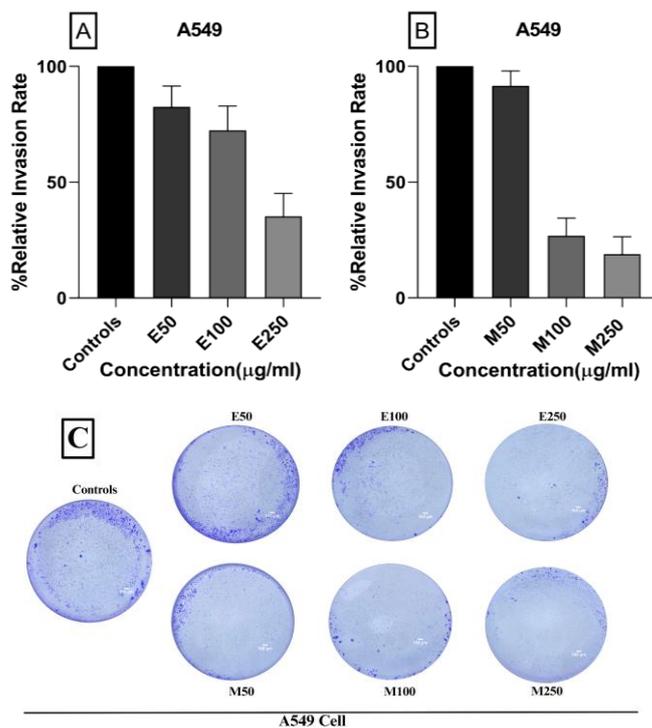
suggesting a dose-dependent inhibitory effect (**Figure 4(B)**). The results from the Transwell invasion assay suggest that *M. speciosa* extracts exhibit a concentration-dependent inhibitory effect on A549 cell invasion, with higher concentrations (100 - 250  $\mu\text{g/mL}$ ) demonstrating suppression, while lower concentrations (M50 and E50) exhibit weaker inhibition. This pattern is consistent with previous findings on natural compounds affecting epithelial-mesenchymal transition (EMT) and matrix metalloproteinase (MMP) regulation. For instance, Ent-Caprolactin C, a marine-derived compound, effectively inhibited invasion via suppression of TGF- $\beta$ -induced EMT, downregulating N-cadherin,  $\beta$ -catenin, and vimentin, key proteins associated with metastatic progression [19]. Similarly, extracts from *Akebia trifoliata* and *Premna puberula* have demonstrated inhibitory effects on MMP-2 and MMP-9, reducing the invasiveness of A549 cells in a

dose-dependent manner [20,21]. The inhibitory trend observed in *M. speciosa* extracts, particularly at 100 µg/mL (M100 and E100), suggests a potential mechanism involving EMT or MMP regulation, as found in these previous studies. Interestingly, the increased invasion at 50 µg/mL (M50 and E50) raises the possibility that *M. speciosa* extracts may enhance cell migration at sub-inhibitory concentrations, a phenomenon reported for certain plant-derived compounds that modulate cytoskeletal dynamics and focal adhesion kinase (FAK) signaling [22]. This effect could be attributed to partial activation of pro-migratory pathways, which might lead to enhanced motility before inhibitory concentrations are reached. This phenomenon has also been observed in studies of low-dose TGF-β, where transient EMT activation enhances invasion before complete EMT suppression at higher concentrations [23]. At the highest tested concentrations (250 µg/mL, M250 and E250), the moderate inhibition observed compared to the 100 µg/mL group suggests a saturation effect or possible cytotoxicity-independent mechanisms at play. High concentrations of bioactive plant extracts often induce non-specific cytotoxic effects, which may contribute to reduced invasion independently of EMT inhibition. This is supported by findings in *Premna puberula*, where increasing extract concentrations resulted in a plateau of inhibitory effects rather than a linear decline in invasion [21]. Collectively, these findings indicate that *M. speciosa* extracts exert dose-dependent effects on A549 invasion, with potential involvement of EMT suppression and MMP downregulation at optimal inhibitory concentrations (100 µg/mL). However, the pro-migratory effects observed at lower doses warrant further investigation into the possible activation of FAK, PI3K/AKT, or Rac1 signaling pathways, which are known to facilitate cancer cell motility [24]. Future studies should explore the molecular underpinnings of these effects, particularly focusing on the expression of EMT-related transcription factors (Snail, Twist, and ZEB1), MMP activity, and cytoskeletal remodeling proteins to elucidate the precise mechanisms by which *M. speciosa* influences lung cancer cell invasion.

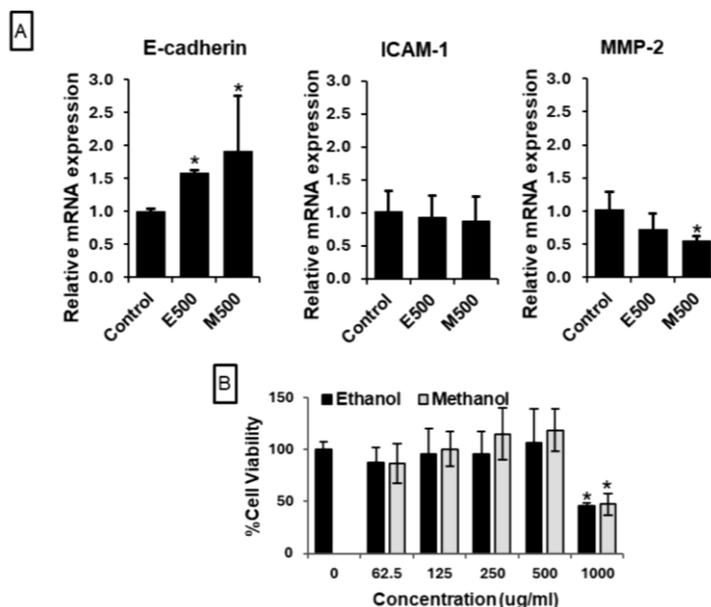
In addition to the phenotypic suppression of A549 cell invasion observed in this study, the underlying mechanisms by which *M. speciosa* exerts its anti-invasive effects remain to be elucidated. Previous investigations have demonstrated that mitragynine, the principal alkaloid constituent of *M. speciosa*, can induce early apoptosis in A549 cells, as evidenced by Annexin V/PI staining [14]. This apoptotic response may contribute to the reduction in invasive potential, independent of classical invasion-related pathways such as EMT or MMP activity. Furthermore, mitragynine has been reported to attenuate cell migration in glioma and neuroblastoma cell lines, suggesting possible interference with cytoskeletal remodeling or motility-associated signaling cascades [16].

#### **Alteration of e-cadherin, ICAM-1 and MMP-2 mRNA expressions**

The genes involved in cell adhesion, invasion, and cancer metastasis include e-cadherin, ICAM-1, and MMP-2 were investigated. The gene expressions of e-cadherin and MMP-2 were altered by both ethanolic and methanolic *M. speciosa* extracts at a concentration of 500 µg/mL-treated for 12 h as shown in **Figure 5(A)**. These findings are associated with the results of migration and invasion assays in A549 cells. Upregulation of e-cadherin mRNA expression has been reported to associate with cancer cell invasion including A549 cells [25] and involved in tumor progression [26,27]. Also, the downregulation of MMP-2 mRNA expression has been reported to suppress adhesion, migration and invasion in A549 cells [28,29]. Interestingly, only methanolic extracts exhibited the downregulation of MMP-2 nor ethanolic extract of *M. speciosa*. However, ICAM-1, which has been reported the highly NF-κB-dependent in A549 cells, was found not significant downregulate in this study [30]. These genes are downstream signals of NF-κB pathway which are widely studied in mechanism of cancer metastasis [31]. Treatment with both ethanolic and methanolic extracts at a concentration of 500 µg/mL for 12 h did not affect cell viability which were shown in **Figure 5(B)**.



**Figure 4** The relative invasion rate of A549 cells treated with *M. speciosa* extracts was determined using the Transwell invasion assay: (A) Cells treated with ethanolic extract (E50, E100, E250) at concentrations of 50, 100, and 250 µg/mL, respectively. (B) Cells treated with methanolic extract (M50, M100, M250) at concentrations of 50, 100, and 250 µg/mL, respectively. Data are expressed as the percentage of invasion relative to the control group (100%). (C) Images were captured under a phase-contrast stereoscope at 10× magnification. Scale bar = 100 µm.



**Figure 5** Ethanolic and methanolic extracts of *M. speciosa* significantly altered the mRNA expression of adhesion molecules on A549 cells. (A) Relative mRNA expressions of E-cadherin, ICAM-1, and MMP-2 in treated-A549 cells for 12 h which were determined by real time RT-PCR. (B) Cell viability of treated-A549 cells for 12 h. Data are presented as mean ± SD from 3 independent experiments (n = 3) \*p < 0.05 vs control.

## Conclusions

This investigation demonstrated that *M. speciosa* extracts exhibit dose-dependent cytotoxic, anti-migratory, and anti-invasive effects against A549 non-small cell lung cancer cells. At optimal concentrations (100 µg/mL) Both methanolic and ethanolic extracts inhibited cell migration and invasion of A549, suggesting potential anti-metastatic properties. The extracts displayed low toxicity toward non-cancerous cells, highlighting their potential as a natural therapeutic agent for lung cancer treatment. These findings support the further exploration of *M. speciosa* for pharmaceutical applications, which could enhance its medicinal value and contribute to the development of alternative cancer treatments.

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## Declaration of Generative AI in Scientific Writing

The authors declared that generative AI tools, including ChatGPT (by OpenAI) and Perplexity, were utilized to support the writing process, specifically for language refinement and grammar correction. These tools were not involved in the generation of original content, interpretation of results, or data analysis. The authors have carefully reviewed the entire manuscript and take full responsibility for its accuracy and conclusions.

## CRedit author statement

**Sasis Phoonket:** Conceptualization/research design, Methodology, Data analysis, Investigation, Data Curation, Writing - Original Draft, Review & Editing/conclusion

**Waranurin Yisarakun:** Conceptualization/research design, Resources, Methodology, Data analysis, Validation, Writing-review & Editing/conclusion, Supervision/criticism.

**Pattaravadee Srikoon:** Investigation, Data analysis, Validation, Writing – review & editing.

**Kulwara Poolpol:** Conceptualization/research design, Resources, Methodology, Data analysis, Validation, Writing - Original Draft, Review & Editing/conclusion, Supervision/criticism, Project administration, Funding acquisition.

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