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Research Paper

# Myristicin Inhibit Invasion and Migration of Melanoma Cells through Suppression of MMP2 and MMP9 Gene Expression

Ni Putu Eka Krisnayanti[1], Silmi Mariya[2,3], Iin Indriawati[2], Hepy Dayana[2], Bella Fatima Dora Zaelani[2], Raymond R. Tjandrawinata[4], Huda Shalahudin Darusman[1,2,3,5*]

[1]Biotechnology Graduate School of IPB University, Kampus IPB Dramaga Bogor, West Java, 16680, Indonesia
[2]Primate Research Center of IPB University, Bogor, West Java, 16151, Indonesia
[3]Primatology Graduate School of IPB University, Kampus IPB Dramaga, Bogor, West Java, 16680, Indonesia
[4]Dexa Laboratories of Biomolecular Science, Jababeka II, Bekasi, West Java, 17530, Indonesia
[5]School of Veterinary Medicine and Biomedicine, IPB University, Kampus IPB Darmaga Bogor, West Java, 16680, Indonesia
*Corresponding author: hudada@apps.ipb.ac.id

**Abstract**
Melanoma is the deadliest type of skin cancer, having a high mortality rate. This cancer has an aggressive nature, is highly invasive, and has the tendency to metastasize. Matrix metalloproteinases (MMPs) are essential in that process, especially MMP2 and MMP9. Their expression is upregulated during metastasis progression. Myristicin is one example of a compound that can be utilized to target MMP 2 and MMP 9 in melanoma. This research concerns the activity of myristicin to inhibit melanoma cell invasion and migration by suppressing MMP2 and MMP9 gene expression. The MTT assay in this study demonstrated that myristicin exhibited strong cytotoxic activity against melanoma cells. This compound works in a dose-dependent manner by inhibiting cell migration and invasion. The invasion test was performed using the transwell assay, whereas the migration test was performed using the wound healing assay. The invasion assay results were consistent with MMP2 and MMP9 gene expression. These two genes were analyzed using the RT-qPCR technique. It has been demonstrated that low gene expression in melanoma cells inhibits cell invasion. In contrast, higher MMP2 and MMP9 gene expression was associated with an increase in the number of invasive cells on average. However, MMP2 and MMP9 in excessive expression and uncontrolled activity impair the ability of melanoma cells to form a monolayer sheet to cover wound gaps. This condition significantly reduced the migration rate and percentage of wound closure.

**Keywords**
Invasion, Melanoma, Migration, MMP2 & MMP9, Myristicin

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## 1. INTRODUCTION

Melanoma is the deadliest type of skin cancer. This cancer is aggressive, highly invasive, and tends to metastasize, so its mortality rate is high (Hessler et al., 2020; Shrikhande et al., 2015). Metastatic melanoma leads to a poor prognosis. The 5-year relative survival rate of localized and regional melanoma are 98% and 64%, respectively. However, it falls to 23% for metastatic melanoma (stage IV) (Rebecca et al., 2020). Melanoma, which has disseminated to distant sites, is incurable in most cases (Goding, 2017; Maverakis et al., 2015). There is no treatment yet capable of targeting cell invasion and metastasis (Ju et al., 2018).

The progression during metastasis allows melanoma cells to invade the dermis layer and enter the circulatory system. This process involves the important role of matrix metalloproteinases (MMPs). These enzymes degrade the basement membrane (BM) and the extracellular matrix (ECM), then promote melanoma cells migration and invasion (Braeuer et al., 2014; Zhou et al., 2015). There are two types of MMPs, most of which are regulated in invasive tumor cell evolution. They are MMP2 and MMP9 (Shay et al., 2015). MMP2 is highly expressed during melanoma progression and may indicate an increase in invasion potential in melanoma (Tsung et al., 2008). MMP9 expression was also elevated, and in some tumors, such as melanoma, it becomes a marker of aggressiveness. (Falzone et al., 2016; Zhang et al., 2016).

MMPs as the target of therapeutics are expected to be a promising strategy for curing melanoma. Synthetic MMP inhibitors have been shown to have promising preliminary results in inhibiting tumor aggressiveness. Some of them made it to clinical trials, while others were discontinued prematurely ow-

ing to either a lack of advantages or their undesirable effects (Egeblad and Werb, 2002; Napoli et al., 2020; Villareal et al., 2018). Natural compounds with biological activity against cancer cells can be utilized as an alternative in melanoma treatment by targeting MMP 2 and MMP 9. They have relatively low or no toxicity, generally (Gam et al., 2021). One example of a compound with that potential is myristicin. This compound is a natural alkyl benzene derived from herbs and spices like nutmeg, cinnamon, fennel, coriander, cloves, dried celery, and star anise (Bao and Muge, 2021).

Myristicin is known to possess anticancer properties. Recent research has demonstrated that myristicin can inhibit cell proliferation and lead to apoptosis in gastric cancer cells (Song et al., 2023). Nazar and Ayyappan (2024) reported that myristicin can generate cell cycle arrest at the G1/S phase and apoptosis in breast cancer cells. Several earlier studies found that myristicin can inhibit cancer cell migration and invasion. This compound can reduce migration and invasion in liver carcinoma (Chunyan et al., 2020) as well as in colon cancer (Bao and Muge, 2021).

The other molecular activities induced by myristicin are that this compound can inhibit gene expression of MMP2 and MMP9. Chunyan et al. (2020) identified these activities in colon cancer. Research from Lee et al. (2007) showed that myristicin can decrease MMP9 gene expression in inflammatory cells. Luo et al. (2022) also discovered that myristicin's molecular activity in human vascular smooth muscle cells suppresses MMP9 gene expression. All those activities of myristicin are considered to impede the migration and invasion of the cells tested. So far, no studies on the effect of myristicin on melanoma invasion and migration capacity have been conducted. It is essential to carry out this research as the first step in assessing the potential of myristicin against melanoma. This research aims to examine myristicin's ability to inhibit invasion and migration of melanoma through suppressing MMP2 and MMP9 gene expression. This research is expected to open the path for the development of novel melanoma therapies.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

The materials used for this research are B16-F10 melanoma cell lines (ATCC CRL-6475), Dulbecco's modified Eagle medium (DMEM, high glucose; Gibco™, USA), fetal bovine serum (FBS Brazil origin, EU approved, Sterile filtered; HiMedia, India), penicillin-streptomycin (Corning®, USA), phosphate buffer saline (PBS; Vivantis, Malaysia), myristicin (09237 Supelco®; Sigma-Aldrich, USA), thiazolyl blue tetrazolium bromide (MTT) solution (Sigma-Aldrich, USA), ethanol, transwell insert (CLS3422 Transwell® polycarbonate membrane cell culture inserts; Corning®, USA), MaxGel ECM (E0282 Sigma-Aldrich, USA) solution, crystal violet (HiMedia, India), GF-1 total RNA extraction kit, (Vivantis, Malaysia), SensiFAST cDNA synthesis kit (Bioline, Meridian Life Science, USA), SsoFast™ EvaGreen® Supermix (Bio-Rad, USA).

### 2.2 Instrumentation

This research utilized the following instruments: class IIA biological safety cabinet (Nuaire, USA), $CO_2$ incubator (Thermo L-80XP, USA), inverted microscope (Nikon, Japan), microplate reader (Bio-Rad, Singapore), nanodrop (Thermo, USA), and thermal cycler machine (Thermo, USA).

### 2.3 Methods

#### 2.3.1 MTT Assay

B16-F10 cells at a density of $5\times10^3$ cells per well were cultured in 96-well microplates. They were incubated for 24 h and then treated with myristicin at concentrations of 0.01, 0.05, 0.1, and 0.5 mM. Cells without myristicin served as the control. The incubation period was 48 hours at 37°C. Subsequently, 10 $\mu$L of 0.5 mg/mL MTT solution was applied to each well and incubated at 37°C for 4 hours. Formazan crystals in each well were dissolved in 100 $\mu$L of 96% ethanol. The absorbance was measured by a microplate reader at 595 nm. The percentage value of cell viability was calculated and analyzed further through linear equations using Microsoft Excel. This equation is then used to determine the %inhibitory concentration of 50% ($IC_{50}$). All experiments were performed in triplicate as independent experiments.

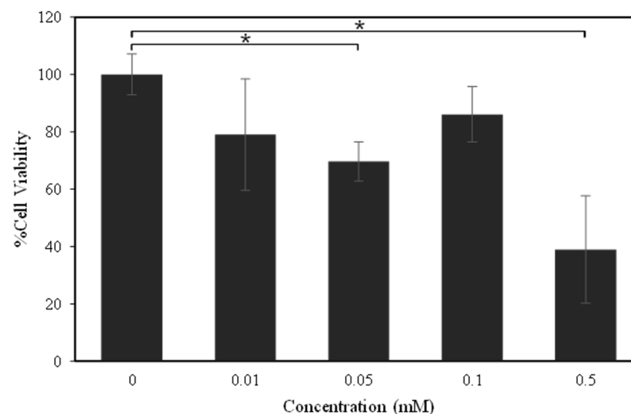


**Figure 1.** Cytotoxic Activity of Myristicin Against Melanoma Cells (B16-F10) After 48 Hours of Incubation in the MTT Assay. Each Point Represents the Mean ± SEM of Three Independent Experiments ($*p < 0.05$ vs. Control Group)

#### 2.3.2 Transwell Assay

The transwell assay was used to detect the cell invasion capacity of melanoma. This procedure refers to He et al. (2022) and Justus et al. (2014), with some adjustments. The transwell insert was coated with 50 $\mu$L of MaxGel ECM solution. Cell culture medium was added to 24-well plates, and the insert was placed on top of the well. Myristicin solutions with concentrations of 0.4 (1× $IC_{50}$), 0.2 (0.5× $IC_{50}$), and 0.1 (0.25× $IC_{50}$) mM were prepared in reduced serum medium (DMEM + 1% FBS +

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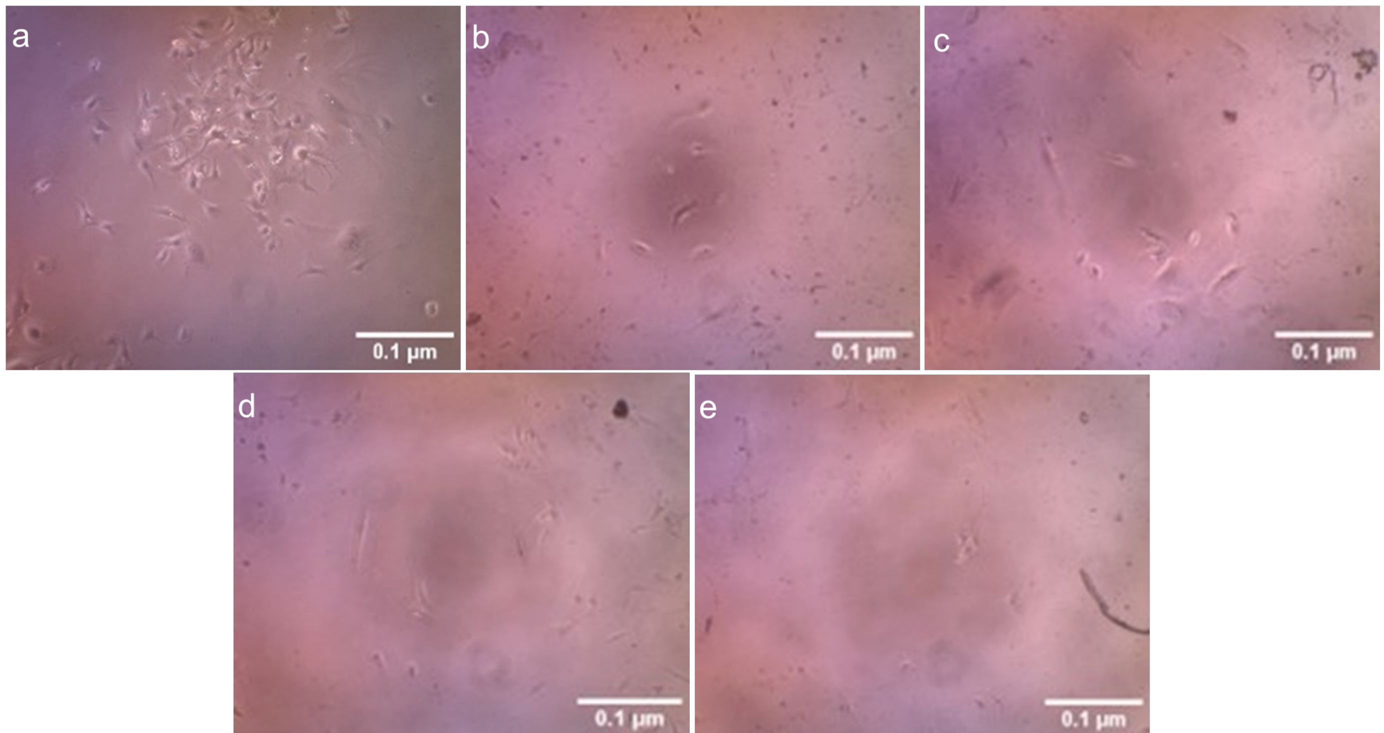


**Figure 2.** Morphology of B16-F10 Cells After 48 Hours of Incubation in the MTT Assay. Myristicin Was Administered to Cells at Various Concentrations (a: 0 mM; b: 0.01 mM; c: 0.05 mM; d: 0.1 mM; e: 0.5 mM; Scale = 0.1 $\mu$m)

1% penicillin-streptomycin). B16-F10 cells were resuspended in those solutions (cell density of $1 \times 10^6$ cells/mL), and 100 $\mu$L of each solution was plated into the insert. B16-F10 cells, which were resuspended in a reduced serum medium without myristicin, were considered a control. Incubation was conducted for 24 hours in the incubator, and then non-invading cells were removed from the insert. The invasive cells were fixed with 70% ethanol and stained with 0.2% crystal violet. The invasive cells were observed under an inverted microscope, and documentation was taken in four fields of view. Invasive cells will appear blue, and their total number will be counted and then averaged.

#### 2.3.3 Wound Healing Assay

The cell migration assay was conducted through the wound healing assay, which refers to the procedure described by He et al. (2022) and Justus et al. (2014) with several modifications. Myristicin solutions were prepared in a reduced serum medium, just like the transwell assay before. B16-F10 cells were seeded in 12-well plates at a density of $5 \times 10^5$ cells per well and incubated for 24 hours. The cell culture medium was subsequently changed with PBS. The monolayer cells of each well were scratched vertically in a straight line with 200 $\mu$L pipette tips, then wiggled gently to remove loose cells. The PBS was discarded, and the cells were treated with myristicin solutions. A reduced serum medium without myristicin is used as a control. All experiments were performed in duplicate as independent experiments. Images of the wound healing process were photographed digitally at 0, 4, 8, and 24 hours. Measurements of the area of the wound gap from those images were carried out using the Image J software. The results of this measurement were used to analyse the migration rate (Jonkman et al., 2014) and the percentage of wound closure (Suarez-Arnedo et al., 2020).

#### 2.3.4 Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

MMP2 and MMP9 gene expression analyses were performed on cells derived from the migration test. Following the reference manual, the total RNA was extracted from the cells. The RNA concentration was measured using a nanodrop. Briefly, cDNA was synthesized in reverse transcription according to the manufacturer's instructions. Subsequently, cDNA was used for amplification. We performed qPCR using GAPDH as an endogenous control. Ct values were read by Quantstudio Design and Analysis Desktop Software. The relative gene expressions were quantified in Microsoft Excel using the delta-delta Ct ($\Delta\Delta$Ct) method.

#### 2.3.5 Data Analysis

The percentage of cell viability, migration rate, percentage of wound closure, and average number of invasive cells were all tested using the unpaired T-test. The IBM SPSS Statistics 26 application was used to run this test. A $p$-value $< 0.05$

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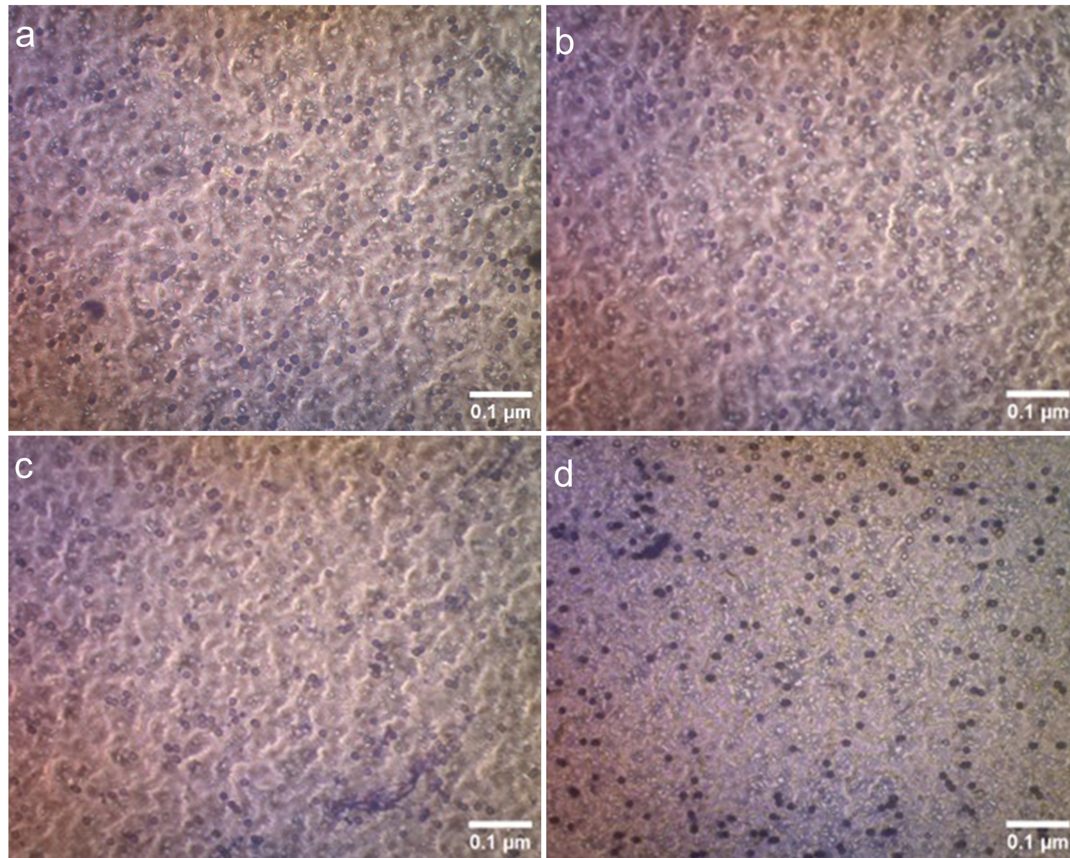


**Figure 3.** Invasive Cells of B16-F10 That Penetrate the MaxGel Layer on the Transwell Insert After 24 Hours of Incubation. The Invasive Cells Appear Blue. Cells Were Treated with Myristicin at Several Concentrations (a: 0 mM; b: 0.1 mM; c: 0.2 mM; d: 0.4 mM; Scale = 0.1 $\mu$m)

was considered statistically significant. The percentage of cell viability is presented as the mean ± standard error of the mean (SEM). The migration rate, wound closure percentage values, and average number of invasive cells are presented as the mean ± standard deviation (SD).

## 3. RESULTS AND DISCUSSION

### 3.1 Cytotoxic Activity of Myristicin

A cytotoxic assay on B16-F10 cells was carried out using the MTT assay to determine the potential toxicity of myristicin. Cells treated with myristicin solution after 48 hours of incubation generally had lower viability than the control. The unpaired T-test revealed that the concentrations of 0.05 mM and 0.5 mM significantly differed from the control. Meanwhile, there was no statistically significant difference in values between 0.01 mM and 0.1 mM concentrations compared to the control (Figure 1). The lowest cell viability value was obtained at a myristicin concentration of 0.5 mM after 48 hours of incubation. Lee et al. (2005) discovered that myristicin significantly reduced the viability of SK-N-SH cells (human neuroblastoma cells) compared to control at a concentration of ≥ 0.5 mM for 24 and 48 hours of exposure periods. Another research by Martins et al. (2014) reported that at 500 $\mu$M (0.5 mM), myristicin reduced the viability of K562 cells (human leukemia cells) by more than 20% after 24 and 48 hours of incubation. Based on these two studies, it was also stated that myristicin had a dose-dependent effect on the viability of the cells tested. This means the effect on the cells tested can change when the dose changes.

Further data analysis was performed to determine the $IC_{50}$ value of myristicin against melanoma cells. The cytotoxicity level of a test compound based on the $IC_{50}$ value is categorized as very strong if it is $< 10$ $\mu$g/mL. If the $IC_{50}$ value ranges between 10 and 100 $\mu$g/mL, then it is classified as strong. A compound with an $IC_{50}$ value of 100–500 $\mu$g/mL is regarded as moderately cytotoxic (Savitri et al., 2023; Weerapreeyakul et al., 2012). Furthermore, Machana et al. (2011) define a test compound as not having cytotoxic activity if the $IC_{50}$ value exceeds 500 $\mu$g/mL. In this study, the $IC_{50}$ value was 0.39 mM ($R^2$ = 0.78), equivalent to 75 $\mu$g/mL. This finding aligns with Martins et al. (2014), who discovered that the $IC_{50}$ value of myristicin against human leukemia cells was 368 $\mu$M or 0.368 mM. Based on the findings of this study, myristicin has strong cytotoxic activity against melanoma cells.

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The morphology of B16-F10 cells was also observed using an inverted microscope at 32× magnification. Under an inverted microscope, the B16-F10 cells in the control group appeared spindle-shaped and resembled epithelial-like cells. There are also pseudopodia-like protrusions that are cytoplasmic extensions (Figure 2a). Meanwhile, B16-F10 cells treated with different doses of myristicin exhibited morphological alterations. They predominantly appeared to be elongated and slimmer than the control cells. The cytoplasmic extensions also become longer and thinner (Figure 2b-e). These results are similar to those published by Cui et al. (2017) in their study using genistein compound. Normal B16-F10 cells are spindle-shaped, while the treatment group exhibited elongated and slim pseudopodia-like protrusions.

### 3.2 Effect of Myristicin on The Invasion of B16-F10 Cells

The transwell assay was used in this study to investigate the inhibition of melanoma cell invasion. Blue cells indicate invasive cells that have penetrated the MaxGel layer (Figure 3). Melanoma cells are highly aggressive and metastasize rapidly. These characteristics are primarily related to their migration and invasion abilities (Chang et al., 2023; de Franca et al., 2021). This study revealed that myristicin, in a dose-dependent way, can inhibit the invasion of melanoma. Myristicin with concentrations of 0.1 and 0.2 mM had a lower average number of invasive cells compared to the control. These two concentrations can reduce the invasion ability of melanoma cells. The outcomes changed when the myristicin concentration was 0.4 mM. The average number of cells successfully invading the membrane layer on the insert was higher than at the control and other test doses (Table 1). According to statistical analysis, the number of invasive cells decreased significantly only at a myristicin concentration of 0.2 mM compared to the control. In the meantime, when 0.4 mM myristicin was given, there was a notable rise in the quantity of invasive cells. This treatment dose promotes melanoma cell invasiveness (Figure 4).

**Table 1.** The Average Number of Invasive Cells

| Concentration (mM) | The Average Number of Invasive Cells ± SD |
|---|---|
| 0 (control) | 223.33 ± 3.055 |
| 0.1 | 214.00 ± 7.000 |
| 0.2 | 212.67 ± 5.686 |
| 0.4 | 239.33 ± 9.018 |

### 3.3 Effect of Myristicin on The Migration of B16-F10 Cells

Observation of the wound gap area under an inverted microscope is shown in Figure 5. Cells migrate to fill the wound gap, and Figure 6a exhibits B16-F10 cell migration rates ($V_{migration}$). Myristicin treatment at doses of 0.1 mM and 0.4 mM differed significantly from the control. Another treatment with 0.2 mM myristicin showed no significant difference from the control. This could be due to a variance in cell migration rate

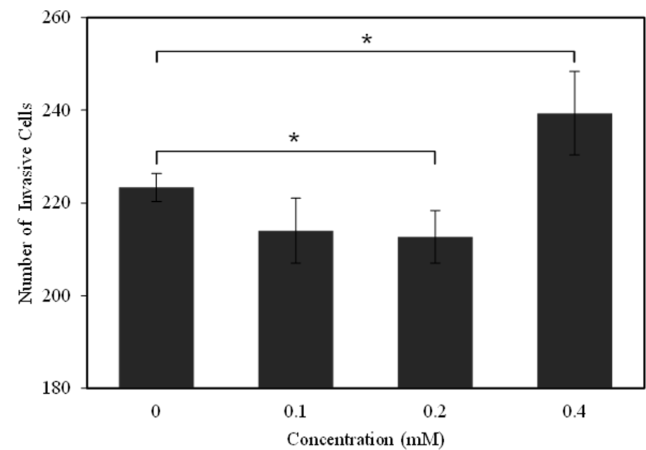


**Figure 4.** Effect of Myristicin on the Invasion of B16-F10 Cells. The Number of Invasive Cells After 24 Hours of Incubation. Each Point Represents the Mean ± SD of Four Fields of View (*$p < 0.05$ vs. Control Group)

values between replications in each treatment group. Jonkman et al. (2014) stated that cells at the edge frequently move at different speeds into the wound gap and do not form a regular monolayer. This condition has an impact on the wound gap measurement results. Their wound closure ability is also affected by their migration rate. At all myristicin test concentrations, the percentage of wound closure was smaller than the control. A significant decline occurred at a concentration of 0.4 mM (Figure 6b).

### 3.4 Effect of Myristicin on MMP2 and MMP9 Gene Expression of B16-F10 Cells

The effect of myristicin on the gene expression of MMP2 and MMP9 in melanoma cells was measured using the RT-qPCR method. Treatment with 0.1 and 0.2 mM concentrations can downregulate the expression of the MMP2 and MMP9 genes. At a concentration of 0.4 mM, the results were divergent, with an increase in MMP2 and MMP9 gene expression (Figure 7a-b).

The invasion assay results aligned with the MM2 and MMP9 gene expression analyses. Melanoma cells with high MMP2 and MMP9 gene expression were followed by an increase in the average number of invasive cells. On the other hand, it has been shown that low gene expression of MMP2 and MMP9 suppresses melanoma cells invasion. These findings pointed out that higher gene expression of MMP2 and MMP9 could allow more cells to invade the transwell membrane. MMP2 and MMP9 may disrupt the MaxGel layer, enabling cells to pass through the transwell membrane pore.

MMP2 can degrade type I and type IV collagen, as well as other ECM components such as gelatin and fibronectin. This enzyme facilitates $\alpha v\beta 3$ integrin binding to cleaved ECM

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0 h 4 h 8 h 24 h

0 mM

0.1 mM

0.2 mM

0.4 mM

**Figure 5.** Observation of the Wound Gap Area at 0, 4, 8, and 24 hours in a Wound Healing Assay of B16-F10 Cells Treated with Different Concentrations of Myristicin (Scale = 0.1 $\mu$m)

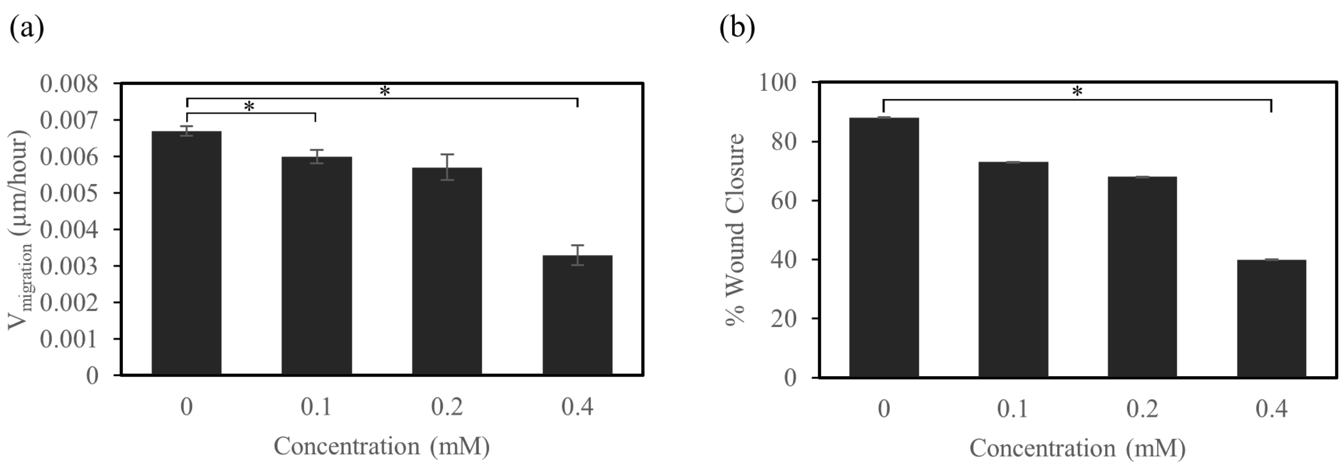


**Figure 6.** Effect of Myristicin on the Migration of B16-F10 Cells. The Migration Rate of B16-F10 Cells (a). The Percentage of Wound Closure After 24 Hours of Incubation (b). Each Point Represents the Mean ± SD of Two Independent Experiments (*$p < 0.05$ vs. Control Group)

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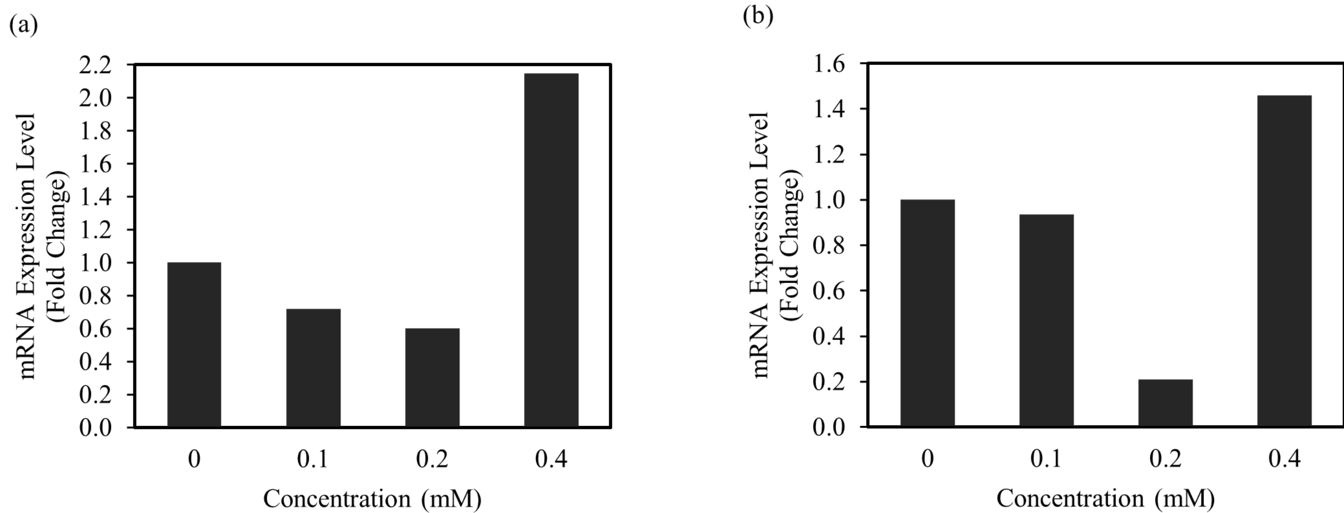


**Figure 7.** Effect of Myristicin on MMP2 (a) and MMP9 (b) Gene Expression in B16-F10 Cells

components, allowing tumor cells to migrate (Bastian et al., 2017; Napoli et al., 2020). MMP9 can also degrade type IV collagen and release ECM-bound angiogenic factors like fibroblast growth factor-basic (FGF-b) and vascular endothelial growth factor (VEGF) (Cabral-Pacheco et al., 2020). Moreover, MMP9 is able to cleave E-cadherin, a cell adhesion molecule, thus allowing cancer cells to leave their original sites (Augoff et al., 2022).

Mills et al. (2002) reported on the effect of downregulating the MMP2 gene on melanoma cells. According to the study, reducing MMP2 expression can suppress the invasiveness of melanoma cells. Tsung et al. (2008) conducted research in which they knocked down the MMP2 gene, decreasing melanoma cell invasion ability. Zhao et al. (2014) also stated in their study that melanoma cells with decreased MMP2 gene expression have a lower invasion ability. Furthermore, Ji et al. (2015) found that inhibiting MMP2 and MMP9 gene expression at the protein level, as well as their enzymatic activity, can reduce melanoma cell invasion. Choi et al. (2017) also demonstrated that suppressing MMP2 and MMP9 gene expression at the mRNA and protein levels can inhibit melanoma cell invasion. Another study by Villareal et al. (2018) also discovered that suppressing the expression of the MMP2 and MMP9 genes, along with the CD44 gene, could significantly inhibit melanoma cell invasion. This study also included *in vivo* testing. Downregulation of the MMP2 and MMP9 genes significantly inhibits melanoma cell metastasis to the lungs.

A surprising finding from the wound healing assay was that myristicin significantly reduced the migration rate and percentage of wound closure at a concentration of 0.4 mM. Excessive expression and uncontrolled activity of MMP2 and MMP9 may impair melanoma cells' ability to form a monolayer sheet to cover wound gaps. Those conditions may affect their interactions and environmental state. Several previous studies have proven MMPs' influence on cell migration and wound healing. These enzymes are frequently found at excessive levels in chronic wounds. Uncontrolled proteolytic activity of MMPs can impair wound healing by degrading growth factor receptors on the cell surface and interfering with cell adhesion molecules. MMPs have also been shown to breakdown growth factors, fibronectin, and other proteins involved in wound healing (Krejner et al., 2016; Sabino and auf dem Keller, 2015; Shi et al., 2012).

MMPs are critical molecules for tissue remodeling and regeneration. Although MMPs are required for proper wound healing, an excess of them or a mismatch between MMPs and their natural inhibitors can impede the healing process (Krejner et al., 2016). Shi et al. (2012) demonstrated *in vitro* that elevated levels of MMP1, MMP2, and MMP9 significantly inhibit keratinocyte cell migration. Keratinocytes don't spread but rather form dense colonies of isolated cells. This condition is assumed to be the underlying cause of wound healing issues. Sabino and auf dem Keller (2015) discovered that increased MMP2 and MMP9 levels correlate with nonhealing chronic wounds. The wound develops to a state of increased ECM degradation, altered cytokine profile, and growth factor degradation, resulting in retarded or null wound healing.

## 4. CONCLUSIONS

Myristicin, in a dose-dependent manner, has the potential to inhibit melanoma cell invasion and migration. The suppression in MMP2 and MMP9 gene expression caused by myristicin exposure was comparable to the decrease in the average number of invasive cells. This state is attained at 0.1 and 0.2 mM concentrations. Excessive expression of MMP2 and MMP9 not only generated more invasive cells but also impaired melanoma

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cells' ability to cover wound gaps. This result is obtained at a concentration of 0.4 mM.

## 5. ACKNOWLEDGMENT

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## REFERENCES

Augoff, K., A. Hryniewicz-Jankowska, R. Tabola, and K. Stach (2022). MMP9: A Tough Target For Targeted Therapy For Cancer. *Cancers*, **14**(7); 1847

Bao, H. and Q. Muge (2021). Anticancer Effect Of Myristicin On Hepatic Carcinoma And Related Molecular Mechanism. *Pharmaceutical Biology*, **59**(1); 1126–1132

Bastian, A., L. Nichita, and S. Zurac (2017). Matrix Metalloproteinases In Melanoma With And Without Regression. In *The Role Of Matrix Metalloproteinase In Human Body Pathologies*. InTech

Braeuer, R. R., I. R. Watson, C. J. Wu, A. K. Mobley, T. Kamiya, E. Shoshan, and M. Bar-Eli (2014). Why Is Melanoma So Metastatic? *Pigment Cell & Melanoma Research*, **27**(1); 19–36

Cabral-Pacheco, G. A., I. Garza-Veloz, C. Castruita-De la Rosa, J. M. Ramirez-Acuña, B. A. Perez-Romero, J. F. Guerrero-Rodriguez, N. Martinez-Avila, and M. L. Martinez-Fierro (2020). The Roles Of Matrix Metalloproteinases And Their Inhibitors In Human Diseases. *International Journal Of Molecular Sciences*, **21**(24); 9739

Chang, K. F., H. C. Lai, S. C. Lee, X. F. Huang, Y. C. Huang, T. E. Chou, C. Y. Hsiao, and N. M. Tsai (2023). The Effects Of Patchouli Alcohol And Combination With Cisplatin On Proliferation, Apoptosis And Migration In B16F10 Melanoma Cells. *Journal Of Cellular And Molecular Medicine*, **27**(10); 1423–1435

Choi, E. O., E. J. Cho, J. W. Jeong, C. Park, S. H. Hong, H. J. Hwang, S. K. Moon, C. G. Son, W. J. Kim, and Y. H. Choi (2017). Baicalein Inhibits The Migration And Invasion Of B16F10 Mouse Melanoma Cells Through Inactivation Of The Pi3K/Akt Signaling Pathway. *Biomolecules And Therapeutics*, **25**(2); 213–221

Chunyan, D., N. He, L. Zhu, L. Fan, F. Yi, T. Wang, and W. Deng (2020). The Mechanism Of Myristicin Inhibiting Proliferation, Migration And Invasion Of Colon Cancer Cell Lines. *Modernization Of Traditional Chinese Medicine And Materia Materia-World Science And Technology*, **22**(4); 907–913

Cui, S., J. Wang, Q. Wu, J. Qian, C. Yang, and P. Bo (2017). Genistein Inhibits The Growth And Regulates The Migration And Invasion Abilities Of Melanoma Cells Via The FAK/Paxillin And MAPK Pathways. *Oncotarget*, **8**(13); 21674–21691

de Franca, M. N. F., R. G. Isidório, J. H. O. Bonifacio, E. W. P. dos Santos, J. F. Santos, F. M. Ottoni, W. de Lucca Junior, R. Scher, R. J. Alves, and C. B. Corrêa (2021). Anti-Proliferative And Pro-Apoptotic Activity Of Glycosidic Derivatives Of Lawsone In Melanoma Cancer Cell. *BMC Cancer*, **21**(1); 662

Egeblad, M. and Z. Werb (2002). New Functions For The Matrix Metalloproteinases In Cancer Progression. *Nature Reviews Cancer*, **2**(3); 161–174

Falzone, L., R. Salemi, S. Travali, A. Scalisi, J. A. McCubrey, S. Candido, and M. Libra (2016). MMP-9 Overexpression Is Associated With Intragenic Hypermethylation Of MMP9 Gene In Melanoma. *Aging*, **8**(5); 933–944

Gam, D. H., J. H. Park, J. H. Kim, D. H. Beak, and J. W. Kim (2021). Effects Of Allium Sativum Stem Extract On Growth And Migration In Melanoma Cells Through Inhibition Of VEGF, MMP-2, And MMP-9 Genes Expression. *Molecules*, **27**(1); 21

Goding, C. (2017). Translation Reprogramming Key Determinant Of Melanoma Invasion. *Oncology Times*, **39**(6); 1–8

He, D., Z. Ma, K. Xue, and H. Li (2022). Juxtamembrane 2 Mimic Peptide Competitively Inhibits Mitochondrial Trafficking And Activates ROS-Mediated Apoptosis Pathway To Exert Anti-Tumor Effects. *Cell Death And Disease*, **13**(3)

Hessler, M., E. Jalilian, Q. Xu, R. N. Reddy, V. Vasquez-Montes, Z. Szekely, and S. Lin (2020). Biological And Biophysical Underpinnings Of Cell-Cell Fusion And Heterokaryon Formation. *Journal Of Cellular Physiology*, **236**(2); 941–956

Ji, B. C., Y. P. Hsiao, C. H. Tsai, S. Chang, S. Hsu, H. Liu, Y. Huang, J. Lien, and J. Chung (2015). Cantharidin Impairs Cell Migration and Invasion of A375.S2 Human Melanoma Cells by Suppressing MMP-2 and -9 Through PI3K/NF-kB Signaling Pathways. *Anticancer Research*, **35**(2); 729–738

Jonkman, J. E. N., J. A. Cathcart, and F. Xu (2014). An Introduction to the Wound Healing Assay Using Live-Cell Microscopy. *Cell Adhesion & Migration*, **8**(5); 440–451

Ju, R. J., S. J. Stehbens, and N. K. Haass (2018). The Role of Melanoma Cell-Stroma Interaction in Cell Motility, Invasion, and Metastasis. *Frontiers in Medicine*, **5**; 307

Justus, C. R., N. Leffler, M. Ruiz-Echevarria, and L. V. Yang (2014). In Vitro Cell Migration and Invasion Assays. *Journal of Visualized Experiments*, **88**; e51046

Krejner, A., L. Malgorzata, and G. Tomasz (2016). Matrix Metalloproteinases in the Wound Microenvironment: Therapeutic Perspectives. *Chronic Wound Care Management and Research*, **3**; 29–39

Lee, B. K., J. H. Kim, J. W. Jung, J. W. Choi, E. S. Han, S. H. Lee, K. H. Ko, and J. H. Ryu (2005). Myristicin-Induced Neurotoxicity in Human Neuroblastoma SK-N-SH Cells. *Toxicology Letters*, **157**(1); 49–56

Lee, K., C. M. Lee, I. D. Jung, Y. Jeong, S. Chun, H. Park,

© 2024 The Authors. 

I. Choi, S. Ahn, Y. Shin, S. Lee, S. Yeom, J. Kim, and Y. Park (2007). GATA-3 is a Key Factor for Th1/Th2 Balance Regulation by Myristicin in a Murine Model of Asthma. *Journal of Life Science*, **17**(8); 1090–1099

Luo, L., H. Liang, and L. Liu (2022). Myristicin Regulates Proliferation and Apoptosis in Oxidized Low-Density Lipoprotein-Stimulated Human Vascular Smooth Muscle Cells and Human Umbilical Vein Endothelial Cells by Regulating the PI3K/Akt/NF-kB Signalling Pathway. *Pharmaceutical Biology*, **60**(1); 56–64

Machana, S., N. Weerapreeyakul, S. Barusrux, A. Nonpunya, B. Sripanidkulchai, and T. Thitimetharoch (2011). Cytotoxic and Apoptotic Effects of Six Herbal Plants Against the Human Hepatocarcinoma (HepG2) Cell Line. *Chinese Medicine*, **6**; 1–8

Martins, C., C. Doran, I. C. Silva, C. Miranda, J. Rueff, and A. S. Rodrigues (2014). Myristicin from Nutmeg Induces Apoptosis via the Mitochondrial Pathway and Down Regulates Genes of the DNA Damage Response Pathways in Human Leukaemia K562 Cells. *Chemico-Biological Interactions*, **218**; 1–9

Maverakis, E., L. Cornelius, G. Bowen, T. Phan, F. Patel, S. Fitzmaurice, Y. He, B. Burrall, C. Duong, A. Kloxin, H. Sultani, R. Wilken, S. Martinez, and F. Patel (2015). Metastatic Melanoma – A Review of Current and Future Treatment Options. *Acta Dermato Venereologica*, **95**(5); 516–524

Mills, L., C. Tellez, S. Huang, C. Baker, M. Mccarty, L. Green, J. M. Gudas, X. Feng, and M. Bar-Eli (2002). Fully Human Antibodies to MCAM/MUC18 Inhibit Tumor Growth and Metastasis of Human Melanoma. *Cancer Research*, **62**(17); 5106–5114

Napoli, S., C. Scuderi, G. Gattuso, V. Di Bella, S. Candido, M. S. Basile, M. Libra, and L. Falzone (2020). Functional Roles of Matrix Metalloproteinases and Their Inhibitors in Melanoma. *Cells*, **9**(5); 1151

Nazar, S. S. and J. P. Ayyappan (2024). Mechanistic Evaluation of Myristicin on Apoptosis and Cell Cycle Regulation in Breast Cancer Cells. *Journal of Biochemical and Molecular Toxicology*, **38**(6); e23740

Rebecca, V. W., R. Somasundaram, and M. Herlyn (2020). Pre-Clinical Modeling of Cutaneous Melanoma. *Nature Communications*, **11**(1); 2858

Sabino, F. and U. auf dem Keller (2015). Matrix Metalloproteinases in Impaired Wound Healing. *Metalloproteinases in Medicine*, **2**; 1–8

Savitri, R. I., H. A. Nuha, and F. Rifki (2023). Antioxidant, Cytotoxic Activity and Protein Target Inhibition of Ethyl Acetate Fraction Melinjo Seed (Gnetum gnemon L.) by In Vitro and In Silico Studies on HeLa Cervical Cancer Cells. *HAYATI Journal Bioscience*, **30**(5); 864–873

Shay, G., C. C. Lynch, and B. Fingleton (2015). Moving Targets: Emerging Roles for MMPs in Cancer Progression and Metastasis. *Matrix Biology*, **44-46**; 200–206

Shi, L., S. Ramsay, R. Ermis, and D. Carson (2012). *In Vitro* and *In Vivo* Studies on Matrix Metalloproteinases Interacting with Small Intestine Submucosa Wound Matrix. *International Wound Journal*, **9**(1); 44–53

Shrikhande, S. S., D. S. Jain, R. B. Athawale, A. N. Bajaj, P. Goel, Z. Kamran, Y. Nikam, and R. Gude (2015). Evaluation of Anti-Metastatic Potential of Cisplatin Polymeric Nanocarriers on B16F10 Melanoma Cells. *Saudi Pharmaceutical Journal*, **23**(4); 341–351

Song, J., X. Xu, S. He, N. Wang, Y. Bai, Z. Chen, B. Li, and S. Zhang (2023). Myristicin Suppresses Gastric Cancer Growth via Targeting the EGFR/ERK Signaling Pathway. *Current Molecular Pharmacology*, **16**(7); 712–724

Suarez-Arnedo, A., F. Torres, C. Clavijo, P. Arbeláez, J. C. Cruz, and C. Muñoz-Camargo (2020). An Image J Plugin for the High Throughput Image Analysis of In Vitro Scratch Wound Healing Assays. *PLoS One*, **15**(7); e0232565

Tsung, A. J., O. Kargiotis, C. Chetty, S. S. Lakka, M. Gujrati, D. G. Spomar, D. H. Dinh, and J. S. Rao (2008). Downregulation of Matrix Metalloproteinase-2 (MMP-2) Utilizing Adenovirus-Mediated Transfer of Small Interfering RNA (siRNA) in a Novel Spinal Metastatic Melanoma Model. *International Journal of Oncology*, **32**(3); 557–564

Villareal, M. O., Y. Sato, K. Matsuyama, and H. Isoda (2018). Daphnane Diterpenes Inhibit the Metastatic Potential of B16F10 Murine Melanoma Cells In Vitro and In Vivo. *BMC Cancer*, **18**(1); 1–11

Weerapreeyakul, N., A. Nonpunya, S. Barusrux, T. Thitimetharoch, and B. Sripanidkulchai (2012). Evaluation of the Anticancer Potential of Six Herbs Against a Hepatoma Cell Line. *Chinese Medicine (United Kingdom)*, **7**; 15

Zhang, T., K. Dutton-Regester, K. M. Brown, and N. K. Hayward (2016). The Genomic Landscape of Cutaneous Melanoma. *Pigment Cell & Melanoma Research*, **29**(3); 266–283

Zhao, K., L. Wei, H. Hui, Q. Dai, Q. D. You, Q. L. Guo, and N. Lu (2014). Wogonin Suppresses Melanoma Cell B16-F10 Invasion and Migration by Inhibiting Ras-Medicated Pathways. *PLoS One*, **9**(9); e106458

Zhou, L., K. Yang, T. Andl, R. R. Wickett, and Y. Zhang (2015). Perspective of Targeting Cancer-Associated Fibroblasts in Melanoma. *Journal of Cancer*, **6**(8); 717

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