In vitro anticancer activity of *Carthamus tinctorius* extract against non-small cell lines (A549)

Nalinee Pradubyat[1*], Thanapat Songsak[1], Fameera Madaka[2], Suchada Jongrungruangchok[3]

[1]Department of Pharmacology, College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand
[2]Drug and Herbal Product Research and Development Center, College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand
[3]Department of Pharmaceutical Chemistry, College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand
E-mail: nalinee.p@rsu.ac.th

Abstract

The most common type of cancer in the world is pulmonary cancer. Even though various chemotherapeutic medications are used to treat cancer, cancer cells might develop drug resistance. As a result, further research into innovative cancer drugs is still required. This study aims to determine the cytotoxic and antimigration activities of medicinal plant extracts against pulmonary cancer cells, which have become increasingly recognized due to their anticancer benefits, owing to significant advances in the phytochemical study of plants. In this study, *Carthamus tinctorius* was extracted using 90% ethanol and studied in terms of cytotoxic and antimigration activities via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and scratch assays. The plant extract was incubated at an increasing concentration (0 - 320 µg/mL) for 48 hours against pulmonary cancer cells (A549) for both assays. In this study, 5-fluorouracil (5-FU) was used as a positive control. The results showed that *C. tinctorius* extract exhibited anticancer activity with a half inhibitory concentration of 26.38 ± 0.983 µg/mL. In addition, the plant extract showed the potential to inhibit cancer cell migration, demonstrating a significant 40% inhibition of migration in A549 non-small cell lung cancer cells. In conclusion, *C. tinctorius* extract exhibited potent anticancer and antimigration activity against pulmonary cancer. Further studies regarding the mechanism of action are warranted.

Keywords**:** *Anticancer activity, Antimigration, Cytotoxic activity, Carthamus tinctorius, MTT assay, Scratch assay*

1. Introduction

Lung cancer stands as the most prevalent form of cancer globally, both in terms of incidence and mortality rates (Siegel, Miller, Wagle & Jemal, 2023). Non-small cell lung cancer (NSCLC), encompassing adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, constitutes approximately 85% of all lung cancer cases (Siegel et al., 2023). This subgroup is particularly notorious for its poor prognosis, underscoring the urgency for effective treatment strategies (Siegel et al., 2023). The cornerstone of therapy for patients with NSCLC has traditionally been platinum-based compounds, notably cisplatin (D'Addario et al., 2005; Lin et al., 2021). Despite its widespread use, the overall 5-year survival rate for NSCLC patients undergoing treatment with platinum-based regimens is dismally low at 16%. Furthermore, platinum-based treatments are often associated with higher toxicity levels compared to non-platinum-based regimens, exacerbating the challenge of managing the adverse effects inherent in cancer chemotherapy (Dukaew et al., 2020; Yu et al., 2020).

Given these constraints, there is a pressing need to explore and identify novel anticancer agents that can enhance efficacy while simultaneously reducing the toxicity of platinum-based chemotherapy in the treatment of NSCLC. This pursuit is critical to improving the therapeutic outcomes and quality of life for

patients afflicted with this formidable disease. In this context, the exploration of natural compounds, such as *Carthamus tinctorius* (safflower), emerges as a promising avenue. Traditionally, safflower has been utilized for its diverse medicinal properties, including its potential application as an anticancer agent. Ongoing research into the antiproliferative and antioxidant mechanisms of *C. tinctorius* against lung cancer cell lines, particularly in the context of NSCLC, holds significant promise for augmenting the current arsenal of cancer therapeutics (Yu et al., 2020). By delving into the potential of such natural remedies, the scientific community aims to uncover safer, more effective treatment modalities that could revolutionize the approach to lung cancer care, particularly in light of the challenges posed by conventional platinum-based therapies (Yu et al., 2020).

C. tinctorius, commonly known as safflower, is not only well-known for its vibrant hues as a natural food colorant but also revered for its multifaceted medicinal properties. Historically, *C. tinctorius* has been employed to manage a range of health conditions, including hypertension, oxidative stress, and cardiovascular issues, by inhibiting blood clots, dilating blood vessels, and serving as a neuroprotective agent. Moreover, its role as an immunosuppressive cancer drug underscores its potential in the realm of oncology (Alahmadi, Alharbi, Ravindran, & Saravanan, 2023; Li, Li, He, & Yang, 2022). The link between oxidative processes and the onset of cancer is well-documented, with oxidative stress playing a pivotal role in cancer development and progression (Alahmadi et al., 2023; Li et al., 2022; Wu, Cai & Gao, 2021).

Building on this foundation, the present study ventures further into the antioxidant mechanisms of *C. tinctorius*, aiming to unravel its anticancer potential in lung cancer cells. This exploration is timely and pertinent, as natural remedies and their constituents are increasingly recognized for their complementary role in mitigating the side effects associated with conventional chemotherapy. The antioxidant properties of natural compounds offer a dual benefit by directly inhibiting cancer cell growth and ameliorating oxidative damage, thus offering a holistic approach to cancer management.

2. Objectives

1) To evaluate the cytotoxic activity of *C. tinctorius* extract
2) To investigate the antimigration potential of *C. tinctorius* extract
3) To compare the cytotoxic efficacy of *C. tinctorius* extract with 5-fluorouracil (5-FU)

3. Materials and Methods

Plant Extraction

Carthamus tinctorius (safflower) was subjected to an extraction process using 90% ethanol as the solvent. The plant material was macerated in an ultrasonic sonicator for 45 minutes, a procedure that was repeated three times to ensure thorough extraction. Following the sonication process, the resultant mixture was filtered to separate the liquid extract from the plant residues. The filtered extract was then concentrated by removing the ethanol under reduced pressure using a rotary evaporator, yielding the final extract for further analysis.

Cell Line Maintenance

The cytotoxic effects of the *C. tinctorius* extract were evaluated on non-small cell lung cancer (NSCLC) cell line A549. These cell lines were cultured in RPMI 1640 medium, enriched with 10% fetal bovine serum (FBS) to provide essential growth factors and 1% penicillin/streptomycin to prevent bacterial contamination. The cultures were maintained under standard cell culture conditions, with the growth medium refreshed every two days to sustain optimal cell growth and viability.

Cytotoxic Activity Testing

The MTT assay, a widely used method for assessing cell metabolic activity as an indicator of cytotoxicity, was employed to evaluate the antiproliferative effect of the *C. tinctorius* extract on the A549 cell line. Cells were seeded at a density of 1×10^4 cells per mL in 96-well plates, with each well containing 100 µL of the growth medium, and allowed to adhere for 24 hours. Subsequently, the cells were treated with the extract at varying concentrations (0, 10, 20, 40, 80, 160, 320 µg/mL) for 48 hours. Additionally, 5-fluorouracil (5-FU) was used as a positive control at concentrations ranging from 0 to 60 µM. Following treatment, 10 µL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well, and the plates were incubated for an additional 3-4 hours to allow for the formation of formazan crystals. The medium was then carefully removed, and the formazan crystals were solubilized in 100 µL of MTT stop solution (0.2% dimethyl sulfoxide, DMSO). The absorbance of the resulting solutions, which is directly proportional to the number of viable cells, was measured at 570 nm using a Benchmark Plus microplate reader (Bio-Rad, United States). The half-maximal inhibitory concentration (IC_{50}) values, indicating the concentration required to inhibit 50% of cell growth, were calculated from the absorbance data. All experiments were conducted in triplicate to ensure reliability, and the data were statistically analyzed using Microsoft Excel.

Antimigration Activity Testing

For the evaluation of antimigration activity, A549 cells were seeded at a high density of 2 × 10^5 cells per well in 6-well plates to achieve confluency. Once confluent, a scratch was introduced into the monolayer of cells using a 200 µL pipette tip to simulate a wound. The cells were then treated with the *C. tinctorius* extract at the determined concentrations (0 – 20 µg/mL). The migration of cells into the wound area was monitored and photographed at specified time intervals to assess the ability of the extract to inhibit cell migration and wound closure. This assay mimics the process of cancer cell migration and invasion, providing insights into the potential antimetastatic properties of the safflower extract.

4. Results and Discussion

The cytotoxic potential of *Carthamus tinctorius* extract (CTE) was quantitatively assessed against A549 lung cancer cell lines using the MTT assay. The assay evaluates cell viability by measuring the metabolic conversion of MTT to formazan by viable cells, thus providing an indirect measure of cell proliferation and viability **(Figure 1)**. The results are presented as the half-maximal inhibitory concentration (IC_{50}), which represents the concentration of the extract required to reduce the viability of the cancer cells by 50% compared to untreated controls. After 48 hours of incubation with various concentrations of CTE, the IC_{50} value for the A549 cell line was determined to be 26.38 ± 0.983 µg/mL **(Table 1)**. This data indicates that CTE has a significant cytotoxic effect on A549 cells, suggesting its potential utility as an anticancer agent for lung cancer treatment. Additionally, 5-fluorouracil was used as a positive control in this study, with an IC50 of 19.41 micromolar (2.52 micrograms/mL) **(Figure 2, Table 1)**, allowing for a comparative evaluation of CTE's efficacy.

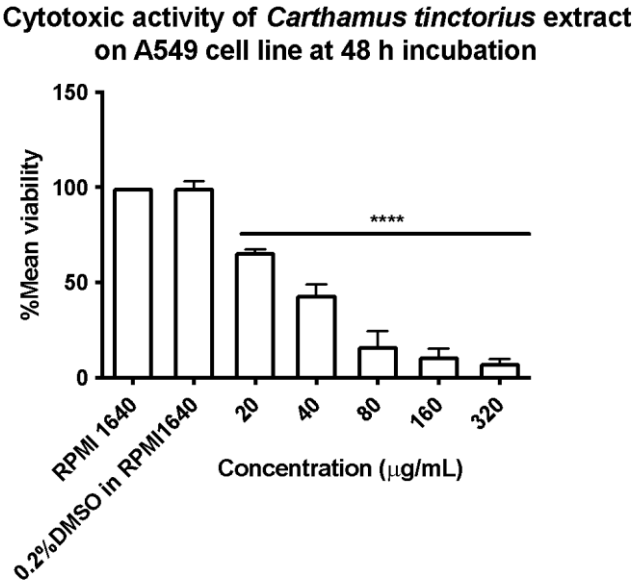

Figure 1: Bar graph illustrating the viability of A549 cells after 48 hours of treatment with *Carthamus tinctorius* extract across various concentrations. As shown, cell viability decreases with increasing concentrations of the extract, indicating a concentration-dependent cytotoxic effect. $^{****}P \leq 0.0001$ represents CTE-treated cells compared to control.

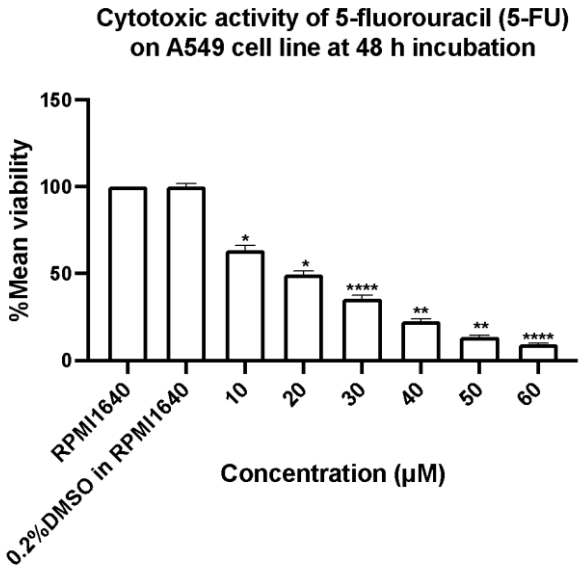

Figure 2: Bar graph illustrating the viability of A549 cells after 48 hours of treatment with 5-fluorouracil (5-FU) extract across various concentrations. As shown, cell viability decreases with increasing concentrations of 5-FU, indicating a concentration-dependent cytotoxic effect. $^{*}P \leq 0.05$, $^{**}P \leq 0.001$, and $^{****}P \leq 0.0001$ represent 5-FU-treated cells compares to control.

Table 1: IC_{50} of *Carthamus tinctorius* extract and 5-FU against A549 cell lines at 48 hours of incubation

Compound	IC_{50} (µg/mL) ± SEM at 48h
C. tinctorius extract	26.38 ± 0.983
5-FU	2.52 ± 0.326

In addition to its cytotoxic effects, the antimigration potential of CTE was evaluated using a wound healing (scratch) assay. This assay simulates a wound or gap and measures the ability of cells to migrate and fill the void, which is an important aspect of cancer metastasis. After 48 hours of treatment with CTE, there was a significant reduction in the migration of A549 cells. The *C. tinctorius* extract showed a notable antimigration effect, inhibiting the migration of A549 non-small cell lung cancer cells by 40% **(Figure 3)**. This result suggests that CTE not only possesses cytotoxic properties but also has the potential to inhibit the metastatic spread of cancer cells. This dual therapeutic action of CTE against pulmonary cancer is noteworthy and highlights its potential utility in cancer treatment strategies that aim to target both tumor growth and metastasis.

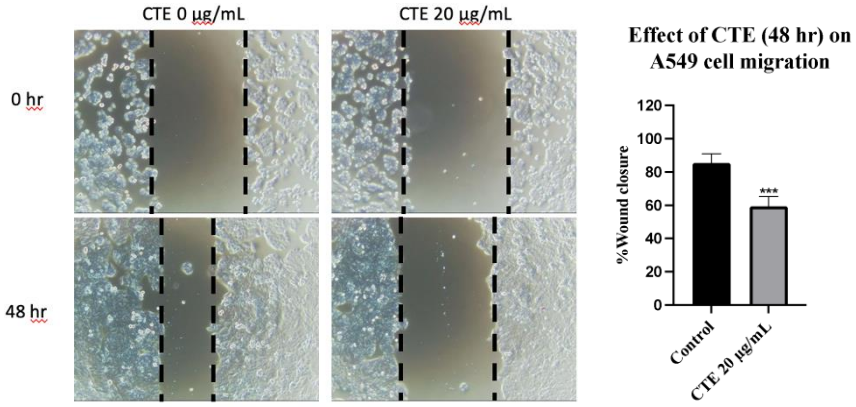

Figure 3: *Carthamus tinctorius* extract inhibited the migration of A549 non-small cell lung cancer cells, the migration of cells into the scratch area between the control group (without treatment) and the group treated with *C. tinctorius* extract, demonstrating a 40% reduction in cell migration due to the extract treatment. $^{***}P \leq 0.001$ represents CTE 20 µg/mL compared to control.

The viability of A549 cells after being treated with CTE for 48 hours demonstrates the concentration-dependent cytotoxic effect of the extract. The reduction in cell viability with increasing concentrations of CTE supports the findings from the IC_{50} determination.

Together, these results underscore the therapeutic potential of *C. tinctorius* extract in the treatment of lung cancer, offering insights into its dual-action capabilities by inducing cell death and inhibiting cell migration, which is critical for effective cancer therapy.

In this study, it is imperative to contextualize the significant cytotoxic and antimigration effects observed with CTE on A549 non-small cell lung cancer cells within the broader landscape of cancer research and treatment. The observed IC_{50} value of 26.38 ± 0.983 µg/mL on lung cancer cells. CTE exhibits a potent cytotoxic effect on lung cancer cells. However, CTE was not as potent as 5-FU, highlighting the need for further optimization and investigation of the extract's potential as an adjuvant therapeutic option. This result is consistent with previous studies that highlighted the anticancer properties of plant extracts, attributing them to a variety of mechanisms, including apoptosis induction, cell cycle arrest, and the inhibition of metastasis

(Güner, Kızılşahin, Nalbantsoy, & Karabay Yavaşoğlu, 2020). The cytotoxic activity of CTE adds to this body of evidence, suggesting that compounds within the extract may be interacting with cellular pathways that are pivotal in cancer cell proliferation and survival (Güner et al., 2020).

The antimigration effect, characterized by a 40% reduction in cell migration, likewise points towards the extract's potential to inhibit processes essential for cancer metastasis, such as cell adhesion and antimigration effect (Kurman et al., 2023). This finding is particularly significant given that metastasis is a leading cause of cancer-related mortality, and options to prevent or reduce this process are valuable in cancer treatment (Chen, Wang, Zhang, & Gao, 2020).

The dual action of CTE, both as a cytotoxic and antimigration agent, suggests its potential utility as a multifaceted therapeutic agent. This aligns with the growing interest in multitargeted therapies in oncology, which aim to address the complex nature of cancer by simultaneously targeting multiple pathways involved in the growth and spread of tumors (Li et al., 2022).

While the results are promising, it is important to consider the limitations of the study and the need for further research. *In vitro* findings, such as those presented here, provide critical initial insights but must be followed by in vivo studies to understand the behavior of the extract in a more complex biological environment (Zeng et al., 2022). Additionally, the identification and isolation of active compounds within the CTE would be valuable for elucidating the specific mechanisms of action and for potential development into more targeted therapies.

Overall, the cytotoxic and antimigration activities of CTE against A549 NSCLC cells highlight its potential as a source of novel anticancer compounds. These findings contribute to the expanding research on natural products for cancer therapy, supporting their continued exploration as complementary or alternative therapeutic options. Future studies should focus on translating these in vitro results to in vivo models and ultimately to clinical applications, with the ultimate goal of improving outcomes for patients with lung cancer.

5. Conclusion

The present study demonstrated the significant cytotoxic and antimigration effects of *C. tinctorius* (safflower) extract on A549 non-small cell lung cancer cells. The cytotoxic activity was quantitatively evidenced by an IC_{50} value of 26.38 ± 0.983 µg/mL, indicating the potent ability of the extract to reduce cell viability by 50%. This finding underscores the potential of *C. tinctorius* extract as an effective anticancer agent capable of inducing cell death in lung cancer cells. Furthermore, the antimigration assay revealed that treatment with *C. tinctorius* extract resulted in a 40% reduction in cell migration, highlighting its significant potential to inhibit the metastatic spread of cancer cells. This dual therapeutic action, encompassing both cytotoxic and antimigration effects, positions *C. tinctorius* extract as a promising candidate for further investigation in the context of lung cancer treatment. The implications of these findings are twofold. Firstly, they contribute to the growing body of evidence supporting the therapeutic potential of natural plant extracts in oncology, particularly in the treatment of lung cancer. Secondly, they justify further research to elucidate the underlying mechanisms of action of *C. tinctorius* extract, which could pave the way for the development of novel anticancer drugs with reduced toxicity and enhanced efficacy compared to conventional chemotherapy. Given the ongoing challenges in cancer treatment, including drug resistance and the adverse effects associated with current therapies, the exploration of natural compounds like *C. tinctorius* offers a valuable avenue for discovering new therapeutic strategies. Ultimately, this study provides a foundation for future investigations into the anticancer properties of *C. tinctorius* and its potential integration into comprehensive cancer care protocols.

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