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Potential of *Cananga odorata* and *Citrus limon* essential oils in modulating NOTCH1 signalling for non-melanoma skin cancer treatment

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Abstract

Natural products have drawn increasing attention as potential therapeutic agents, particularly for their anticancer properties. Essential oils (EOs) from *Cananga odorata* (CO) and *Citrus limon* (CL) are well-known for their antibacterial and antioxidant activities, yet their ability to suppress skin cancer cell growth remains uncertain. Whereas NOTCH1 signalling pathway plays an oncogenic role in cancer development due to uncontrolled cell proliferation. This study evaluates the antiproliferative effects of CO and CL EOs on skin cancer cells by examining their impact on NOTCH1 gene expression and protein secretion. Human foreskin fibroblast (HFF-1) and skin carcinoma (A431) cell lines were treated with DMSO-dissolved cisplatin and varying concentrations (125, 250, and 500 µg/ml) of CO or CL EO for 24 hours. Cellular morphology was observed under 20–40x magnification, while qRT-PCR and western blot analyses were conducted to measure NOTCH1 gene expression and protein secretion. In A431 cells, CO EO at 125 µg/ml significantly downregulated *NOTCH1* gene expression compared to untreated cells, with levels lower than those induced by cisplatin. In contrast, CL EO at the same concentration upregulated *NOTCH1* gene expression. At higher EO concentrations, both CO and CL EOs exhibited effects similar to cisplatin, with increased NOTCH1 protein secretion, likely resulting from apoptosis or necrosis-associated membrane leakage. These findings underscore the potential of natural products, particularly CO EO as an anticancer agent via modulation of NOTCH1 signalling. Further studies are essential to unravel the precise mechanisms by which these EOs affect A431 cells, advancing their potential use in skin cancer therapy.

Keywords: *Cananga odorata, Citrus limon, essential oil, NOTCH, skin cancer*

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Introduction

Skin cancer, although less common in Malaysia compared to Western countries, remains a significant health concern. Based on the most recent 2020 data from the World Health Organisation (WHO), Malaysia recorded 247 skin cancer-related deaths, accounting for 0.15% of all fatalities. With an age-adjusted death rate of 0.91 for every 100,000 individuals, Malaysia ranks 139th globally for skin cancer mortality (World Life Expectancy, 2020). Although it accounts for less than 1% of mortality, the rate probably may increase since the awareness of the disease is poor, even among the patients who were already under treatment (Bath-Hextall *et al.*, 2013). The spread and increment of the knowledge of the disease among the population will help to alleviate

the burden of treatment costs, and improve the quality of life (Gordon *et al.*, 2020).

Cancer is a condition where normal cell proliferation becomes uncontrolled with genome alteration (Rashid *et al.*, 2022). Skin cancers are categorized into two main types, which are non-melanoma skin cancer and melanoma. Non-melanoma skin cancers comprise basal cell carcinoma, making up 70-80% of this category, and squamous cell carcinoma (SCC), representing 20% of non-melanoma skin cancers. Even though melanoma only constitutes 4% of skin cancer cases, it is the deadliest type due to its high potential to spread rapidly and unpredictably, especially distanced metastases in advanced stages. It is responsible for 75% of all skin cancer fatalities (Al-Naggar, 2013). In addition, the NOTCH pathway regulates cell proliferation and differentiation, which has been implicated in the pathogenesis of cancer. Hence, hyperactivation of the *NOTCH* gene results in oncogenicity in a few types of cancers, such as breast cancer, T-cell acute lymphoblastic leukemia, SCC, and adenoid cystic carcinoma (Aster *et al.*, 2017).

Many studies reported that the *NOTCH1* gene is involved in skin cancer cells, but its treatment is not without side effects (Ansary *et al.*, 2022; Gellrich *et al.*, 2019). Cisplatin, a platinum-based chemotherapy used in advanced cutaneous SCC, has been associated with acute kidney injury in 20–35% of patients (Fang *et al.*, 2021). Its prolonged presence in plasma, even decades after treatment cessation, contributes to mitochondrial dysfunction, necrosis, apoptosis, and oxidative stress (Ranasinghe *et al.*, 2022). Whereas current studies do not support topical 5-fluorouracil (5-FU) as a standard treatment for cutaneous SCC. Existing reports are limited to case series for 5-FU, with inconsistent follow-up durations and histologic clearance. Moreover, 5-FU often causes prolonged skin irritation, which reduced patient compliance and compromised treatment efficacy (Kim *et al.*, 2018).

Recently, the exploration of natural products as therapeutic alternatives has become an area of interest, with essential oils (EO) demonstrating their potent anticancer properties (Osanloo *et al.*, 2021). For instance, *Cananga odorata* (CO), a naturally growing plant in Asian nations, exhibits antiviral (Nuning *et al*, 2024), antibacterial, antioxidant (Mrani *et al.*, 2024), and soothing properties (Borgonetti *et al.*, 2022). A recent study conducted revealed that the terpene β-caryophyllene, found in CO and other plants, has been recognised for its antitumor activity, particularly against prostate and breast cancers (Sedky *et al.*, 2023).

Similarly, the therapeutic and pharmacological effect of *Citrus* spp. essential oils have been widely reported as possessing antibacterial, antioxidant (Frassinetti *et al.*, 2011), anticancer, and anti-inflammatory properties (Rafique *et al.*, 2020). *Citrus limon* essential oil (CL EO) is proven to have antiproliferative effects, with studies highlighting its superior treatment efficacy against breast and cervical cancer cells compared to other EOs (Osanloo *et al.*, 2021; Othman *et al.*, 2022).

Despite the broad interest in studying essential oils' anticancer effects, the potency of CO and CL EO in inhibiting skin cancer cell proliferation is still lacking. Therefore, this study aims to determine the effect of CO and CL EO on the morphology of normal cell and non-melanoma skin cancer cells, and to investigate the effects of both EOs on *NOTCH1* gene expression and protein secretion in the same cells.

Materials and Methods

Materials

All reagents/instruments were available in the Microbiology-Pharmacology -Physiology (MPP) and Biochemistry Molecular & Proteomic (BMOLEP) laboratory, Department of Basic Medical Sciences, International Islamic University Malaysia (IIUM). Whilst all reagents/chemicals were purchased from Sigma-Aldrich, USA, unless stated otherwise.

Cell culture

The A431 squamous carcinoma and HFF-1 foreskin fibroblast cells were purchased from iCell Bioscience, Inc., Shanghai. These cell lines were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM) (Biosera, France) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin, and were grown in a 75cm^2 flask. The cells were incubated under 5% CO_2 at a temperature of 37°C and subcultured when it was 80-90% confluent according to the standard method.

Cell morphology study and cell harvesting

Cells were seeded at a density of 3.0×10^7 cells/well in 6-well plates in similar culture environment until approximately 90% confluence. The cells were then treated with commercially available CO and CL EO (Plant Therapy, USA) due to their standardized composition and batch consistency, supported by GC-MS profiling. This approach minimizes batch-to-batch variation and ensures reproducibility, which aligns with accepted practices in pharmacological research.

EOs were dissolved in dimethyl sulfoxide (DMSO) and applied at 125, 250, and 500 µg/ml for 24 hours in triplicates. These concentrations were based on previous studies showing cytotoxic effects of citrus EOs within 5–150 µg/ml (Borusiewicz *et al*., 2017). Whereas the in vitro antiproliferative potential of CO EO remains less extensively studied, necessitating broader concentration testing to evaluate its biological activity. The final concentration of DMSO in all treatment conditions was maintained at 0.1%, a level generally regarded as non-toxic for in vitro assays.

Using an inverted light microscope (Olympus, Japan), the morphology of the cells was recorded under 20–40x magnification. For studies on protein and gene expression, adherent cells were harvested using TRIzol (Thermoscientific, USA) and mechanical scraping (Biosera, France). These cell lysates were kept at -80°C. For the protein secretion study, the collected conditioned medium was kept at -20°C.

Total RNA extraction from cell lysate

The total RNA was extracted from HFF-1 and A431 cell lysates in TRIzol using the innuPREP RNA Mini Kit (Analytik Jena, Germany) according to the manufacturer's protocol. Elution buffer was added to the total extracted RNA and was kept at -80°C.

cDNA conversion

The concentration of RNA samples was verified by measuring its absorbance at 260 nm via NanoDrop 1000 Spectrophotometer (Thermoscientific, USA). Using a cDNA conversion kit from BioLine (UK), 1,000 ng of RNA was converted to cDNA and was kept at -20°C.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Predesign *NOTCH1* and *ACTB* primers and probes were purchased from IDTDNA, USA (Table 1). The procedure was performed in a 10 µl reaction composed of Mastermix 2x (IDTDNA, USA), cDNA (1:10 dilution), 20x primers and probes, and RNAse-free water. Samples without transcriptase were used as control reactions. The CFX96 qRT-PCR system (BioRad, USA) was used to conduct the suggested procedure, which involved polymerase activation: 95°C for 3 min; denaturation: 95°C for 15 sec; and annealing: 60°C for 1 min. The CFX96 setting (available from BioRad, USA) was programmed to repeat the amplification up to 50 cycles. All statistical results were analysed by the CFX96 qRT-PCR system. Data are presented as mean ± SEM. A p-value <0.05 was considered statistically significant.

Table 1. Primers Sequences for Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR).

Gene	Primer Sequence		Probe
	Forward (5'-3')	Reverse (5'-3')	
NOTCH1	CGAGGTCAACACAGACGAG	ACAGATGCCCAGTGAAGC	ATGAGTTCCAGTGCGAGTGCCC
ACTB	ACAGAGCCTCGCCTTTG	CCTTGCACATGCCGGAG	TCATCCATGGTGAGCTGGCGG

Protein extraction from cell lysate

Cytosolic protein was extracted from the second filtrate acquired from the innuPREP RNA Mini Kit as described previously (Radzuan, 2020). Briefly, the filtrate was precipitated with 4 volumes of cold acetone at 4°C for 30 min. Protein pellets were obtained by centrifugation at 4000 rpm g at 4°C for 10 min. The supernatant was discarded and 400µl of 100% ethanol was added to the pellet and centrifuged again for 1 minute. The supernatant was removed, and the pellet was air-dried for 10 minutes and resuspended in RIPA extraction and lysis solution (Thermoscientific, USA) before being used for BCA assay. The protein samples were kept at -20°C.

Protein extraction from conditioned medium

The collected conditioned medium was added to four volumes of 100% cold acetone and incubated at -20°C for 1 hour as previously described (Radzuan, 2020). The protein extracts were centrifuged using an ultracentrifuge (Beckman Coulter Life Sciences, USA) at 13000 g, 4°C for 10 min. The supernatant was discarded and the protein pellet was air-dried. The final pellet was suspended in RIPA extraction and lysis buffer and kept at -20°C.

Western blot

Western blotting was used to determine the NOTCH1 protein expression and secretion from HFF-1 and A431-CO or CL EO-treated cells. The primary protein (NOTCH1: A-8, Santa Cruz Biotechnology, USA) and the reference protein (ACTB: C4, Santa Cruz Biotechnology, USA) antibodies were diluted at 1:100, while the secondary antibodies (m-IgGk BP- HRP: sc516102 and m-IgG Fc BP-HRP: sc525409, Santa Cruz Biotechnology, USA) were diluted at 1:1000 for this application. Equivalent concentrations of cytosolic and secreted proteins were denatured in 6x and 2x Laemmli buffer (BioRad, USA) respectively. These two groups of proteins were loaded and separated in 2 different 7% bis-acrylamide gels, with a protein molecular weight marker (BioRad, USA) in the first well of each gel. Electrophoresis was run at 100V for 10 min, followed by 120V for 1 hour.

Subsequently, the proteins were electro-transferred to the PVDF membranes using the Trans-Blot Turbo Kit (BioRad, USA) at 13V for 20 min. After the completion of protein transfer, the PVDF membranes were blocked using a 1% BSA at room temperature for 1 hour. Then, the PVDF membranes were soaked in a working solution that consisted of the primary and reference protein antibodies overnight at 4°C. The following day, the membranes were rinsed and incubated with secondary antibodies for 1 hour at room temperature. The signal development was carried out by using the Chemi-Lumi One Series for HRP (Nacalai Tesque, Japan). Quantification of Western Blotting was done using the

ChemiDoc Imaging System Machine and ChemiDoc XRS software (BioRad, USA).

Results

Cell morphology

Cell morphology was evaluated using light microscopy at two magnifications to optimize visual assessment. A higher magnification (40×) was used for untreated HFF-1 cells to provide a detailed baseline of individual cell morphology. These cells exhibited a typical spindle-shaped appearance, uniform distribution, high confluency, and healthy morphology (Figure 1a).

For the treatment groups, lower magnification (20×) was employed to allow broader visualization of cell population changes across larger areas, including alterations in density, distribution, and morphology. HFF-1 cells treated with DMSO and 125 µg/ml of either CO or CL EO maintained their spindle shape and cell density, indicating negligible or minimal cytotoxic effects at this concentration (Figure 1b, c, f).

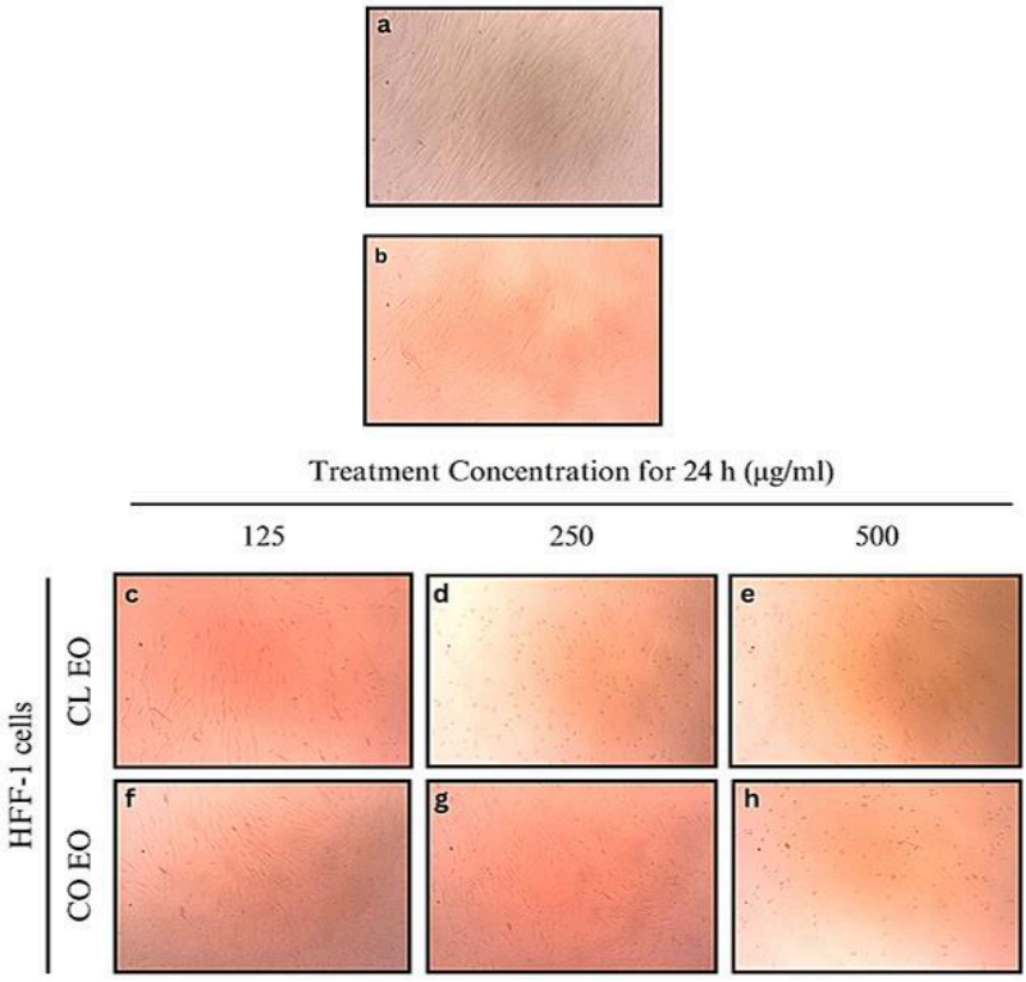

Figure 1. Morphological cell study of HFF-1 cell line. (a) Cells without treatment observed under 40x magnification. (b) Cells treated with DMSO, (c) 125 µg/ml of CL EO, (d) 250 µg/ml of CL EO, (e) 500 µg/ml of CL EO, (f) 125 µg/ml of CO EO, (g) 250 µg/ml of CO EO, and (h) 500 µg/ml of CO EO respectively, and observed under 20x magnification.

In contrast, treatment with 250 and 500 µg/ml of CL EO (Figure 1d, e) resulted in cells adopting a rounded morphology, with decreased cell-to-cell contact and reduced confluency, suggesting signs of cytotoxicity or apoptosis.

A similar response was observed in cells treated with 500 µg/ml of CO EO (Figure 1h), where cell rounding and sparse distribution were evident. These morphological changes indicate a dose-dependent effect of both EOs on cells viability and proliferation.

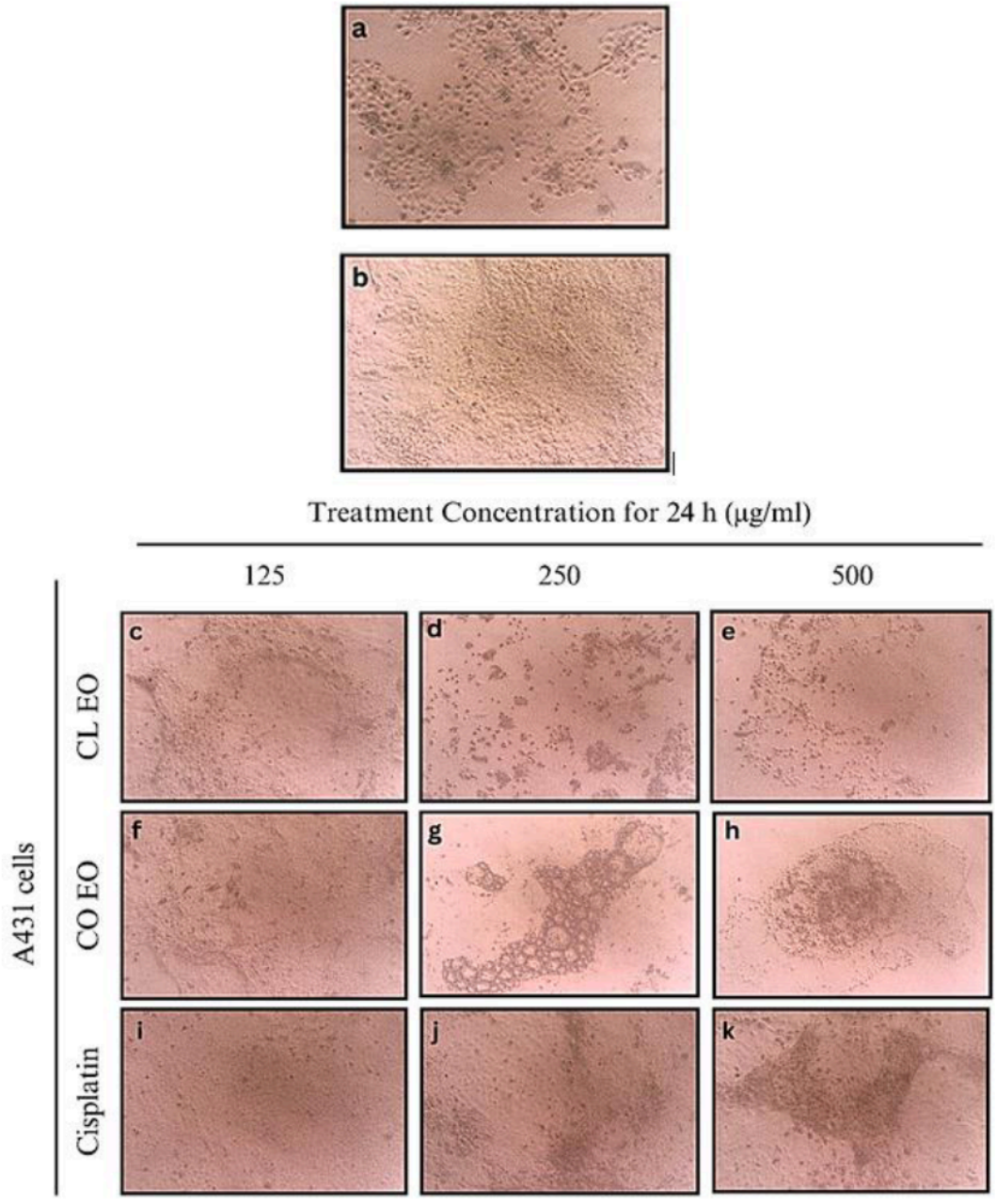

Figure 2. Morphological cell study of A431 cell line. (a) Cells without treatment observed under 40x magnification. (b) Cells treated with DMSO, (c) 125 µg/ml of CL EO, (d) 250 µg/ml of CL EO, (e) 500 µg/ml of CL EO, (f) 125 µg/ml of CO EO, (g) 250 µg/ml of CO E EO, (h) 500 µg/ml of CO EO, (i) 125 µg/ml of cisplatin, (j) 250 µg/ml of cisplatin, and (k) 500 µg/ml of cisplatin respectively, and observed under 20x magnification.

The initial morphology of the untreated A431 cells under 40x magnification appeared round shaped and grew in compact clusters with high confluency (Figure 2a). Morphological features remained unchanged following DMSO exposure, indicating vehicle tolerance (Figure 2b). For treatment groups, a lower magnification (20×) was particularly useful for A431 cells, which exhibit irregular cluster-based growth, enabling clearer visualization of treatment-induced detachment, aggregation, and cell loss.

With increasing concentrations of CL EO, cells exhibited clumped clusters with reduced confluency and cell density (Figure 2c–e). Similar alterations were observed in cells treated with CO EO, where dose-dependent effects included cell swelling, shrinkage, and isolation, particularly at 250 and 500 µg/ml (Figure 2f–h). These changes suggest membrane damage consistent with apoptotic or necrotic processes.

Cisplatin treatment (Figure 2i–k) elicited comparable morphological changes, including cell rounding, hyperpigmentation, and increased granularity. The 20× magnification provided an optimal field of view for evaluating these spatial and morphological alterations in A431 cells.

Gene expression

There is no significant molecular effect to the HFF-1 cells treated with 125 µg/ml CO and CL EO (Figure 3). Interestingly, in contrast to untreated A431 cells, those treated with CO EO showed marked downregulation of *NOTCH1* gene expression as compared to cells treated with 125 µg/ml cisplatin, although it is not statistically significant (p = 0.2) (Figure 4). However, cells supplemented with CL EO revealed insignificant upregulation of *NOTCH1* gene expression ($p > 0.05$), unlike the effects shown by former treatments.

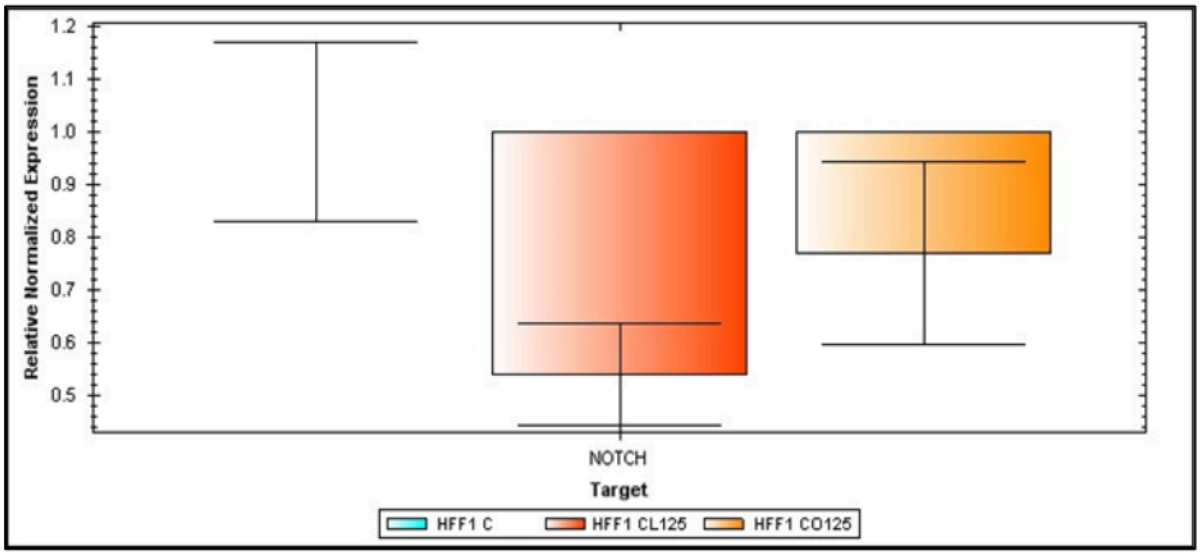

Figure 3. Relative normalized gene expression of NOTCH1 in HFF-1 cells after treatment with CO and CL EO at concentration of 125 µg/ml for 24 hours compared to control.

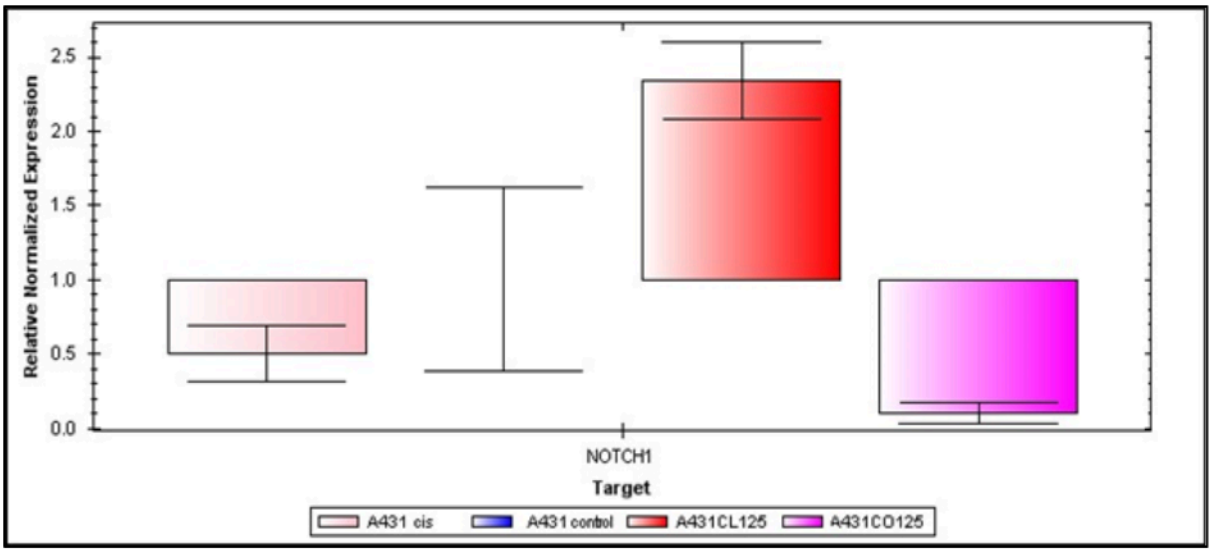

Figure 4. Relative normalized gene expression of NOTCH1 in A431 cells after treatment with cisplatin, CO, and CL EO at concentration of 125 µg/ml for 24 hours compared to control.

Protein expression and secretion

NOTCH1 protein expression could not be completed due to a very low concentration of cytosolic protein extracted from the same cell lysate (less than 0.3 µg/ml). Meanwhile, NOTCH1 protein secretion bands from A431 cells showed increasing intensity with increasing concentration of CO EO, which is almost similar to NOTCH1 protein secretion in cells treated with cisplatin. However, NOTCH1 protein secretion from A431 cells treated with CL EO showed lower protein band intensity (Figure 5).

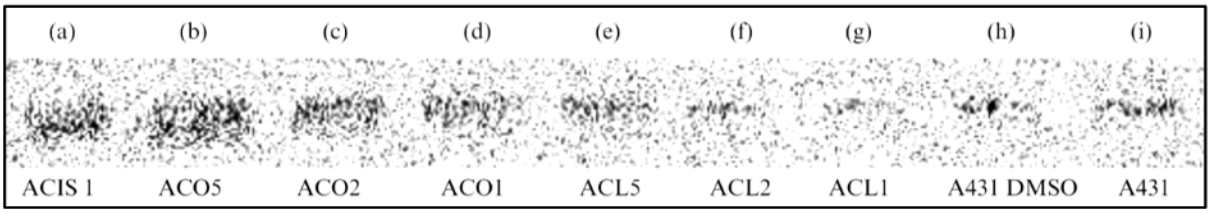

Figure 5. NOTCH1 protein secretion extracted from conditioned media of treated A431 cells. (a) 125 µg/ml cisplatin, (b) 500 µg/ml CO EO, (c) 250 µg/ml CO EO, (d) 125 µg/ml CO EO, (e) 500 µg/ml CL EO, (f) 250 µg/ml CL EO, (g) 125 µg/ml CL EO, and (h) A431 with vehicle (0.1% DMSO), respectively.

The quantitative values were generated by ChemiDoc XRS software and shown in Figure 6. A431 cells treated with 250 µg/ml and 500 µg/ml of CO EO showed marked upregulation of NOTCH1 protein secretion. The latter group displayed the highest NOTCH1 protein secretion, similar to those treated with cisplatin. While cells treated with 125 µg/ml and 250 µg/ml of CL EO showed downregulation of NOTCH1 protein secretion.

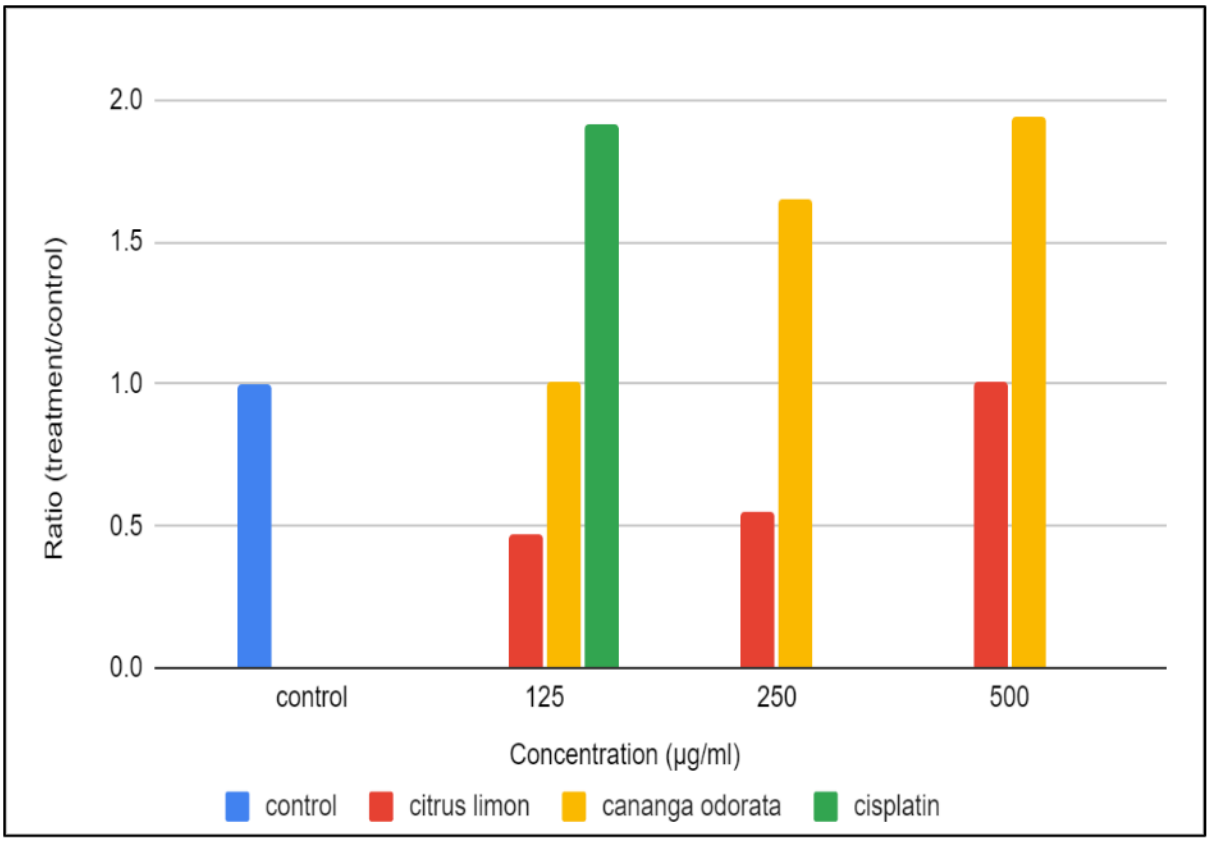

Figure 6. Ratio of NOTCH1 protein secretion on A431 cells.

Discussion

Plant essential oils have been extensively studied for their potential therapeutic properties, including their application in treating skin cancer. To understand the molecular mechanism underlying the effect of plant essential oil on normal skin and skin cancer cells, the morphological cell study, *NOTCH1* gene expression, and western blotting were analysed in this study.

Morphological observations in Figure 1 demonstrated the HFF-1 cells retained their characteristic spindle-shaped appearance when treated with CO and CL EOs at a concentration of 125 µg/ml, suggesting no significant impact on the morphology of normal cells at this level. However, exposure to higher concentrations (250 µg/ml or more) resulted in noticeable morphological changes. As the EO concentration increased, the fibroblast-like structure of the cells progressively transformed into a shrunk, rounded shape, indicating that higher concentrations of EOs disrupted the normal morphology of cells (Kang *et al.*, 2018; Montalvão *et al.*, 2023).

As shown in Figure 3, treatment of HFF-1 cells with 125 µg/ml CO and CL EO did not produce significant changes in *NOTCH1* gene expression compared to the untreated control. This finding, alongside the preserved spindle-shaped morphology and high confluency observed in Figure 1, indicates that low concentrations of CO and CL EOs did not adversely affect normal cells at the molecular or morphological level.

These results aligned with previous findings suggesting the relative safety of certain EO active components, particularly limonene and linalool, major constituents of CO and CL EOs. Herman *et al.* (2015) reported the anticancer potential of both compounds, while also noting their low toxicity profiles. Importantly, these constituents are recognized as safe by several international bodies, including the Research Institute for Fragrance Materials (RIFM) and the U.S.

National Toxicology Program (NTP). Compared to synthetic chemical enhancers, essential oils and their constituents generally present lower toxicity, supporting their potential application as safer transdermal drug delivery agents (Herman *et al*., 2015).

The absence of cytotoxic effects on normal cells is also consistent with studies by Galvão *et al.* (2012), who found no antiproliferative activity of other plant-derived EOs such as *Aloysia gratissima, Baccharis dracunculifolia, Coriandrum sativum*, and *Lippia sidoides*, on normal human cell lines (Galvão *et al*., 2012). Similarly, *Citrus medica* EO also exerted selective cytotoxic effects on human colon cancer cells without significantly affecting non-tumorigenic cells (Fitsiou and Pappa, 2019). These findings collectively support the selective action of certain EOs against cancerous cells, while preserving the viability and integrity of normal cell types.

Given the insignificant morphological and molecular impact of EOs at 125 µg/ml on HFF-1, this concentration was selected to investigate molecular changes in skin cancer cells. However, a precise range of compatible concentrations for normal and cancer cells should be determined before these EOs could be used as an alternative treatment for skin cancer.

Although the morphological appearance of A431 cells treated with 125 µg/ml of CO and CL EOs showed unremarkable changes than the untreated group as shown in Figure 2, this study reported promising findings via *NOTCH1* gene expression. Based on Figure 4, A431 cells supplemented with CO EO caused downregulation of *NOTCH1* gene expression, surpassing the effect observed with cisplatin. The antiproliferative effect shown by CO EO is similar to a study done by Gaafar et al. (2022), in which *Raphanus sativus* extract causes downregulation of *NOTCH1* expression in breast and colon adenocarcinoma cell lines, achieving greater reduction than cisplatin chemotherapy. Additionally, other genes associated with cell proliferation, such as *WNT1* and *SIX1*, were also downregulated, which supports antiproliferative properties of the extract (Gaafar *et al.,* 2022).

In our experiment, treatment with CL EO led to an unexpected upregulation of *NOTCH1* gene expression in cancer cells, despite its anticipated antiproliferative role. This observation could suggest that bioactive compounds of CL EO may modulate cancer cell behaviour through other mechanisms such as cell cycle arrest, induction of apoptosis and antiangiogenic effect (Mohamed Abdoul-Latif *et al*., 2023).

Supporting this, Murthy *et al.* (2012) found that citrus-derived flavonoids act as potent inhibitors of colon cancer cells by suppressing inflammatory markers and inducing cell cycle arrest (Murthy *et al*., 2012). Additionally, essential oil from *Citrus limetta* peel demonstrated anti-inflammatory effects towards skin cancer cells by downregulating cytokines such as TNF-α, IL-1β, and IL-6 (Kim and Hong, 2024). Collectively, these studies support the hypothesis that CL EO may influence cancer progression through multifaceted pathways, including modulation of inflammation and signalling pathway alterations, including but not limited to the NOTCH1 axis.

In Figure 6, A431 cells treated with cisplatin and higher concentration of CO EO exhibited an upregulation of NOTCH1 protein secretion. In contrast, A431 cells treated with lower concentration of CL EO demonstrated a downregulation of NOTCH1 protein secretion relative to the control. These results were inconsistent with the observed gene expression levels of *NOTCH1* in A431 cells.

A potential explanation for these discrepancies probably lies in post-transcriptional modifications. Processes such as mRNA stabilization, translation regulation, or protein stabilization could result in elevated or stable NOTCH1 protein levels despite transcriptional downregulation (Wang, 2011). These mechanisms may influence the accuracy of protein secretion data when compared to gene expression findings. Additionally, complex regulatory pathways might

enhance protein secretion to compensate for reduced intracellular NOTCH1 protein levels due to transcriptional downregulation (Su *et al*., 2024). To confirm whether this secretion involves active transport rather than passive leakage, future experiments incorporating brefeldin A, a known inhibitor of protein trafficking from the endoplasmic reticulum to the Golgi apparatus could provide additional clarity to this hypothesis.

Since the study could not determine cytosolic NOTCH1 protein levels, it remains unclear whether the increased secretion observed in A431 cells treated with CO EO is driven by enhanced synthesis, post-transcriptional regulation, or active secretion mechanisms. Alternatively, cell membrane leakage could be a plausible mechanism in response to treatment-induced stress. Cisplatin, a known chemotherapeutic agent, has been shown to induce both necrosis and apoptosis in cultured human lymphoma and renal tubule cells (Sancho-Martinez *et al.*, 2011). This stress response could result in dose-dependent reduction in protein expression, as reported previously (Rathinam *et al.,* 2015). Consequently, the elevated NOTCH1 protein "secretion" in cells treated with cisplatin and CO EO may reflect intracellular protein leakage into the media rather than active extracellular transport.

To distinguish between passive leakage and active secretion, assays such as LDH release to indicate cell necrosis or TUNEL assays to detect DNA fragmentation in apoptotic cells should be employed. Additionally, the presence of housekeeping proteins like GAPDH and actin in the culture media may suggest compromised cell membrane integrity.

Conclusion

This study showed the potential of CO EO as chemotherapy while maintaining the integrity of normal cell lines. In this study, 125 µg/ml concentration of CO and CL EO shown to have no effect on normal cell morphology and *NOTCH1* gene expression as compared to cancer cells. In addition, treatment of 125 µg/ml CO EO showed further downregulation of *NOTCH1* gene expression than 125 µg/ml cisplatin, which could be a potential alternative to current cancer treatment. However, optimization of methods must be done to produce consistent and reproducible results. Both EOs could be further investigated as a potential complementary therapy for cancer via NOTCH1 signalling, in view of their antiproliferative effect. Apart from LDH release and TUNEL assay, cell viability assay may be included in future research to obtain the cytotoxicity of CO and CL EOs on human cells and cancer cells.

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Conflict of Interest

The author declares that the manuscript produced in the absence of any financial or commercial relationships could be construed as a potential conflict of interest.

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