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Exploring the Potential of 5 Commercial Essential Oils to Inhibit the Proliferation of A549 Lung Cancer Cells

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Abstract

Globally, lung cancer stands as the primary cause of cancer-related fatalities, securing a spot among the top 10 in newly reported cases. The late detection of lung cancer is often attributed to its indication through commonplace symptoms like coughing. Seeking alternatives, this research investigated the medicinal potential of certain essential oils (EOs) with a rich history in traditional medicine. The study aimed to assess the effectiveness of 5 EOs — Clary Sage, Frankincense, Marjoram, Myrrh, and Thyme as a halal alternative therapy for lung cancer A549 cell line. Notably, based on these investigations' findings, Myrrh and Thyme emerged as promising candidates, displaying significant capability to inhibit lung cancer cell expansion. Myrrh had the lowest IC_{50} value, 19 µg/mL, followed by Thyme, 45 µg/mL. In optimisation research, Myrrh resulted in 85% inhibition after 72 hours of exposure to 800 µg/mL concentration. Myrrh demonstrated reduced cell generation and growth rates for cytokinetic study and increased cell death rate. In conclusion, this research was designed to explore the cytotoxic effects of EOs on lung cancer cells using the A549 cell line, leading to the identification of a potential alternative source of halal-compliant pharmaceuticals.

1. Introduction

Lung cancer is the leading cause of death worldwide, along with prostate and breast cancers for both genders. Non-small cell lung cancer (NSCLC) is the most common case reported among the cancer cases, accounting for about 85% overall, while the remaining 15 cases belong to small cell lung cancer (SCLC). There are 3 sub-types of NSCLC: (a) adenocarcinoma, (b) squamous cell carcinoma, and (c) large cell carcinoma (Li & Liu, 2023). To this day, the statistical analysis reports that the 5-year relative survival rate for lung cancer is approximately 22%. Overall, from the lung cancer cases, NSCLC's survival rates stand at 26%, while SCLC's is lower, at only 17%. As only 24% of lung cancer diagnoses happen during the localised stage, early detection of lung cancer is rare. If lung cancer could be detected earlier, the 5-year survival rate could improve to 60%. When talking about earlier detection, the symptoms of lung cancer are normally present only at advanced stages. Not only are the symptoms present late, but they are always misinterpreted as normal symptoms or other types of disease just because they are quite common, such as recurrent lung infections, difficulty breathing, chest pain, persistent coughing, and blood in the sputum (Siegel *et al.*, 2022).

The optimal treatment for lung cancer, whether NSCLC or SCLC, is conditional upon factors such as tumour type, stage, and molecular characteristics. In cases of otherwise healthy individuals diagnosed with early-stage NSCLC, the standard approach involves surgery, possibly complemented by chemotherapy, targeted medications, immunotherapy, and/or radiation therapy (Ahern *et al.*, 2021). For advanced-stage NSCLC, common treatments include chemotherapy, targeted medications, and/or immunotherapy (Guo *et al.*, 2022). Early-stage SCLC is typically addressed with chemotherapy, either alone or in conjunction with radiation. In certain instances of early-stage SCLC, prophylactic cranial radiation may be administered to reduce the risk of brain metastases. Individuals with advanced-stage SCLC may undergo chemotherapy with or without immunotherapy (Hiddinga *et al.*, 2021).

Beyond the conventional approaches to lung cancer treatment mentioned earlier, phytochemicals are developing as promising additions to therapeutic agents (Nguyen *et al.*, 2021). Phytochemicals, derived from plants and employed in traditional medicine for an extended period, are gaining attention among researchers for anticancer therapies. Notable examples of plant-based anticancer medications in the market include vinplastine, etoposide, paclitaxel, and camptotecin (Omara *et al.*, 2020). Essential oils (EOs), considered secondary metabolites produced by plants for protection against environmental stresses, are another approach of interest. These aromatic essences, volatile or ethereal oils, can be extracted from various plants (Sharmeen *et al.*, 2021). With a history rooted in traditional medicine, EOs have been attributed to various biological properties over the centuries. Long-term studies suggest their potential beneficial impact in the fight against cancer. Researchers are growing interested in

natural chemicals and plant-based products with halal status, fascinated by the potential anticancer properties these substances may possess. Unlike synthetic pharmaceuticals, these substances maintain their natural identities, demonstrate comparable efficacy, and often have fewer adverse effects (Abd-Rabou & Edris, 2022).

As the global Muslim population continues to rise, there is a corresponding increase in the demand for halal food and products, crucial for devotees of Islam. Pharmaceuticals developed to address specific diseases, particularly cancer, are vital to these requirements. Recognising the necessity for halal products in the Muslim community, researchers have explored alternative substances that can serve as safe raw materials. While certain fundamental components, such as plants, are inherently considered halal, the utilisation of plant species varies due to potential toxicity in some. Hence, experts are increasingly focusing on natural substances, particularly those derived from plants, as potency halal medical regimens for varied ailments and lung cancer. Essential oils emerge as potentially valuable complementary medicinal sources, drawing from their extensive historical use in traditional medicine. Numerous earlier studies have highlighted the anticancer effects of EOs, positioning them as promising candidates in the pursuit of halal medical treatments. This exploration aligns with the growing awareness of the diverse applications of natural substances, emphasising their potential role in meeting the unique needs of the Muslim community.

Remarkably, there is an increasing focus on the pharmacological applications of EOs, particularly their ability to induce apoptosis in various cancer cell lines. The potent antibacterial effects against various pathogens and well-documented anticancer and antioxidant capabilities, especially in EOs derived from medicinal herbs, underscore their potential therapeutic value. Existing literature strongly supports the broad spectrum of anticancer, anti-inflammatory, and antimicrobial attributes associated with EO (Mahboubi, 2020). Clary Sage (*Salvia sclarea*) has been observed to inhibit cancer cell growth and exhibit antioxidant activities (Mahboubi, 2020). Frankincense (*Boswellia sacra*), highlighted in research by Abd-Rabou & Edris (2022), induces apoptosis in lung cancer A549 cell lines. Marjoram (*Origanum majorana*) displays noteworthy potential by suppressing the proliferation of human HT-29 colorectal cancer cells and impeding their ability to form new colonies (Athamneh *et al.*, 2020). Myrrh (*Commiphora myrrha*) showcases cytotoxicity against breast cancer, as Shehata & Elsewedy (2022) reported. Thyme (*Thymus vulgaris*) exhibits a dose-dependent inhibition of cell proliferation, as evidenced by Niksic *et al.* (2021).

This research aims to investigate the promising potential of these EOs as halal alternative therapeutic sources for lung cancer. The diverse effects observed across these oils underscore their complex nature and the broad spectrum of potential applications in cancer treatment.

2. Materials and methods

2.1 Selection of essential oils

The study utilised 5 commercially obtained EOs for evaluation. The assortment included oil from Clary Sage (*Salvia sclarea*), Frankincense (*Boswellia species*), Marjoram (*Origanum majorana*), Myrrh (*Commiphora myrrha*), and Thyme (*Thymus vulgaris*). These EOs were specifically chosen for their reported therapeutic properties.

2.2 Cultivation and maintenance of human cancer cell lines

Human lung carcinoma cell lines (A549) were acquired from the UPM-MAKNA Cancer Research Laboratory (CANRES) and carefully stored in a cryogenic tank equipped with a liquid nitrogen supply. To ensure their viability, the cell lines were cultivated and consistently maintained in a humidified incubator at 37°C within an environment containing 5% CO_2. The subculture process involved utilising a media culture combination comprising Roswell Park Memorial Institute-1640 (RPMI medium) supplemented with 10% fetal bovine serum (FBS). Following the methodology outlined by Abdik (2021), several chemicals, including accutase (cell detachment solution), dimethyl sulfoxide (DMSO), and phosphate buffer saline (PBS), were systematically included within the subculture process.

2.3 Cell proliferation assay

The cytotoxicity of the A549 cell line was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay, a yellow water-soluble tetrazolium salt. This specific assay assessed the viability of cells following exposure to EOs, determining whether they could induce cell death or inhibit cell growth. The MTT assay, adapted to suit the experimental conditions, followed the procedure outlined by Fithrotunnisa *et al.* (2020). Its primary objective was pinpointing the optimal IC_{50} value among the 5 EOs tested on lung cancer cells. The EOs exhibiting the lowest IC_{50} values, calculated based on the percentage of cell viability, were singled out for further optimisation testing. Conducted 3 times, each assay involved 4 replicates for each type of EO. This experiment's positive and negative controls were 0.5% DMSO and untreated cells. The parameters utilised in the test were kept constant across repetitions. The most favourable result among the repeated tests was carefully selected and incorporated into the dataset, ensuring that the data presented reflects the most promising outcome from this experimental series.

In a flat-bottomed 96-well plate, 100 µL of A549 cells were seeded at a density of 5×10^4 cells/well. Before seeding the cells in the 96-well plate, the cells were first counted using a trypan-blue staining assay to ensure the cells' density was fixed to the desired amount, 5×10^4 cells/well. The 96-well plate was then incubated for 24 hours to ensure the cells grew familiarly in the plate and detached to the wall.

After 24 hours of incubation, the EO with a concentration of 4 mg was added to the 96-well plate with 10-fold dilution and triplicated in the plate. The negative control, 0.5% DMSO, was seeded in the same 96-well plate. The final working amount for each well was 200 µL after adding the EO and negative control, DMSO. The cell was treated and incubated for 48 hours in the incubator before being tested with the MTT reagent. After 48 hours, 10 µL of MTT reagent assay was added to the all well in the 96-well plate. After the assay was added, a vibrant purple colour precipitate was formed. The plate was then again incubated in the incubator for 4 hours. After 4 hours of incubation, 100 µL was discarded, and 100 µL of DMSO was added to each well. The plate was then wrapped with aluminium foil and shaken on the 96-well plate shaker for 5 minutes to ensure the solution was mixed properly. All the process handling of the MTT assay part was done under dark conditions. The absorbance was measured and recorded at 570 nm using a microplate spectrophotometer after 5 minutes of

shaking in the shaker. The data of the reading was recorded for analysis.

The optical density (OD) value directly relates to the number of viable cells in the well. As a result, the OD reading from the MTT test will be used to count the number of cell viability after treatment, evaluate how these cells fare compared to the untreated cells, and further count IC_{50}. In other words, this is essential for determining the relationship between cell survival and cell inhibition, which are inversely related.

2.4 Optimisation of essential oils

Following identifying the most promising EO among the 5 types, characterised by the lowest IC_{50} value, treatment parameters were optimized to determine the optimum conditions for the EO's efficacy. The '2 Factorial Design' was employed for this optimisation process, focusing on two key parameters: EO concentration (µg/mL) and incubation time (hour). The Experiment (DoE) design for this optimisation study was meticulously constructed using Design-Expert software (version 13). The factors included "[A] concentration of EO µg/mL" and "[B] incubation time h," while the response variable for the experiment was "cell viability %."

This optimisation study specifically targeted 2 types of EO: Myrrh and Thyme. The concentration of EOs investigated during the optimisation ranged from 200 µg/mL to 800 µg/mL, and the incubation time for treating cancer cells spanned 24 to 72 hours. The cell viability in the percentage of lung cancer cells was quantised as the response in this optimisation study. It was evaluated using the MTT assay, following the methodology presented by Larsson *et al.* (2020), with necessary adjustments regarding the media use and the range of the parameters to suit the experimental conditions. The systematic exploration of these parameters aimed to define the most effective conditions for maximising the EO's impact on lung cancer cells.

The Analysis of Variance (ANOVA), employing a collection of statistical models to examine the variation and statistical significance in the data, was conducted using Design-Expert software (version 13). This analysis allows for assessing the model's overall significance and the significance of its terms. A "Prob > F" value less than 0.05 indicates that the model terms are statistically significant. Additionally, ANOVA enables the assessment of the lack of fit in the model, providing valuable insights into the model's overall reliability and applicability to the experimental data.

2.5 Cytokinetic study

For the cytotokinetic study, both treated and untreated cells were counted to obtain the cell growth profile. For a start, the A549 from cryopreservation was thawed and grown into the culture plate until confluent. The lung cancer cell was subcultured at least two passaging before being inoculated into the T-25 flask with 5 mL of fresh culture media. Fourty (40) flasks were prepared, 20 flasks for treated cells and 20 flasks for untreated cells. At 8 hours of incubation, one flask containing treated cells and one flask containing untreated cells were harvested from the monolayer and proceeded to cell counting with the trypan-blue staining assay. Following the same process, where 8 hours intervals after that, one flask each was harvested and proceeded to cell counting. The cell collection was triplicated for the cell counting process. The numbers of viable and non-viable cells were recorded to plot the growth curve for this growth kinetics study.

3. Results and discussion

3.1 Assessment of the cytotoxicity

The cytotoxic effect of 5 different types of EOs — Clary Sage, Frankincense, Marjoram, Myrrh, and Thyme was studied against the lung cancer A549 cell line. The MTT assay was employed in this investigation to identify the anti-proliferative and cytotoxic capabilities of these selected EOs. The MTT assay was a valuable tool to evaluate cell inhibition and determine the IC_{50} values, figuring out these EOs' potential to influence lung cancer cells' growth and viability.

As represented in Figure 1, increasing the concentration of the EOs resulted in a noticeable adverse impact on cell viability over all examined EOs. Each EO exhibited a distinct pattern in the graph's drop, reflecting variations in cell growth inhibition. As cell vitality lessened, the inhibition of cell proliferation increased. Notably, the cell viability of both Frankincense and Thyme exhibited a similar decreasing trend, although the extent of reduction differed. This decline was gradual, with the overall count remaining consistent.

In contrast, Myrrh demonstrated a more substantial decrease in cell viability than Frankincense or Thyme, followed by a subsequent reduction aligning with both EOs. Clary Sage and Marjoram exhibited a slower decline in cell viability than the other EOs, yet the final count of viable cells reached approximately the same level. At the highest concentration of EOs, both Myrrh and Thyme proved effective in significantly suppressing the proliferation of cell cultures.

The fact that the EO inhibited the maximum number of cells was demonstrated by the lowest number of cell viability, according to the results of these MTT assays. This implies that the higher the number of inhibited cells, the lower the number of viable cells. According to Table 1, the EOs of Frankincense and Thyme showed the lowest percentage of cell viability, 6.1 ± 1.2% for Frankincense and 6.5 ± 0.7% for Thyme. It was shown that the Frankincense inhibited around 93.9 ± 1.2% of the A549 cells, whereas the Thyme inhibited approximately 93.5 ± 0.7% of the A549 cells. The other 3 EOs showed good inhibitory effects as well. Clary Sage inhibited around 88.7 ± 0.6 of the cells, Marjoram inhibited approximately 86.6 ± 1.7%, and Myrrh inhibited approximately 84.2 ± 1.4% of the cells. After being treated with EOs, the cells were inhibited with a positive result; the proportion of inhibited cells was greater than 80%, demonstrating a good cytotoxic impact. The EOs ranked from the highest to lowest cell inhibition: Frankincense, Thyme, Clary Sage, Marjoram, and Myrrh. This ranking is based on their respective effectiveness in inhibiting the proliferation of the A549 lung cancer cell line, with Frankincense demonstrating the highest inhibition and Myrrh showing the lowest among the selected EOs.

In pharmacological research, the IC_{50} serves as a crucial metric, indicating the amount of a drug required to inhibit a biological process by half. This measurement provides insight into the relative effectiveness of an antagonist agent. Schaduangrat *et al.* (2022) discussed a correlation between the IC_{50} value and the potency of EOs. A lower IC_{50} value signifies greater potency, indicating that a smaller substance is needed to produce the desired effect.

The findings from the previous study by Khalil *et al.* (2020) revealed that the extract of *Commiphora molmol* (Myrrh)

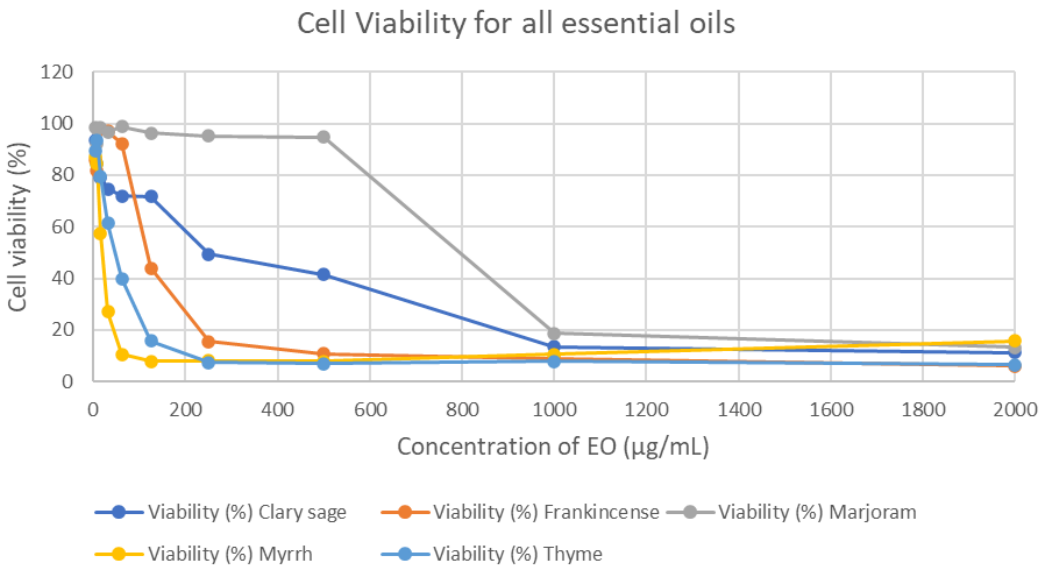

Figure 1: The cell viability (%) graph of lung cancer after being treated with the EO.

Table 1: Cell viability (%), cell inhibition (%), and IC_{50} values for each EO towards lung cancer cell A549 cell line

Essential oil	Cell viability (%)	Cell inhibition (%)	IC_{50} (µg/mL)
Clary Sage (*Salvia sclarea*)	11.3 ± 0.6	88.7 ± 0.6	563 ± 14.4
Frankincense (*Boswellia sacra*)	6.1 ± 1.2	93.9 ± 1.2	119 ± 11.4
Marjoram (*Origanum majorana*)	13.4 ± 1.7	86.6 ± 1.7	752 ± 13.5
Myrrh (*Commiphora myrrha*)	15.8 ± 1.4	84.2 ± 1.4	19 ± 3.3
Thyme (*Thymus vulgaris*)	6.5 ± 0.7	93.5 ± 0.7	45 ± 7.5

exhibited inhibitory effects on human liver cancer (Hep G2), human breast cancer (MCF-7), and colon cancer cell lines (HCT-116). The reported IC_{50} values were 41.52 µg/mL, 10.93 µg/mL, and 19.71 µg/mL, respectively. In the present study, Myrrh demonstrated a similar inhibitory effect on the A549 cell line with an IC_{50} value of 19 µg/mL. Notably, this IC_{50} value falls within the range observed in the previous research studies, highlighting the consistency of Myrrh's inhibitory potential across different cancer cell lines.

Similarly, the previous study by (Niksic *et al.*, 2021) documented the inhibitory effects of *Thymus vulgaris* (Thyme) on non-small cell lung cancer cells (H460) and breast cancer (MCF-7), with IC_{50} values of 68.59 µg/mL and 52.65 µg/mL, respectively. In the current study, Thyme demonstrated an IC_{50} value of 45 µg/mL for treating the A549 cell line. The results indicate that the IC_{50} values are nearly equivalent to those observed in the previous research study, reinforcing the consistent inhibitory impact of Thyme, particularly on lung cancer cells.

Referring to Table 1, Myrrh exhibited the lowest IC_{50} value, specifically 19 µg/mL. Following closely, Thyme had the second-lowest IC_{50} value at 45 µg/mL. Consequently, Myrrh and Thyme were selected for the optimisation study. This step aimed to identify the optimal parameters for the most favourable outcomes in suppressing lung cancer cell proliferation. The selection was supported by their impressive inhibitory effects, as denoted by their low IC_{50} values. To summarise, the EOs that showed the highest cell inhibition to the lowest rank were the Frankincense, Thyme, Clary Sage, Marjoram, and Myrrh.

3.2 Optimisation of essential oils

The optimisation was conducted with two experiments: Myrrh and Thyme. Table 2 shows the result from the design-expert software for both EOs. The 2-factor design generated 16 runs experiment, and based on the results from all the runs, the lowest percentage of cell viability for the Myrrh was 15% with 800 µg/mL for 72 hours, and the highest percentage was 24% with 200 µg/mL for 24 hours. For the Thyme, the lowest percentage of cell viability was 16% with 500 µg/mL for 48 hours, and the highest percentage was 72% with 200 µg/mL for 72 hours.

Based on the data presented in Table 3, the F-values for the models generated by the software are 39.68 for Myrrh and 249.81 for Thyme. As mentioned earlier, P-values lower than 0.05 indicate the significance of the model terms. In this case, both Myrrh and Thyme have P-values less than 0.05, affirming the significance of both EOs in the model. The model components A and B are identified as crucial in this analysis. A significant lack of fit indicates a substantial deviation between predicted and actual values, considered non-significant to attain a fit model. Examining the data in Table 3, the p-values for the lack of fit generated by the software are 0.0755 for Myrrh and 0.1829 for Thyme. As recommended, these p-values are greater than 0.05, signifying that the lack of fit is statistically insignificant. The non-significant lack of fit suggests that the predicted values align well with the actual data, affirming the adequacy and reliability of the model for both Myrrh and Thyme.

Table 2: Cell viability (%) of lung cancer cells treated with Myrrh and Thyme

Run	Factor 1 A:Concentration (µg/mL)	Factor 2 B:Incubation time (hours)	Response 1 Cell viability (%) (Myrrh)	Response 2 Cell viability (%) (Thyme)
1	200	72	20 ± 0.5	72 ± 1.2
2	200	24	24 ± 0.3	59 ± 1.1
3	200	72	17 ± 0.2	69 ± 1.4
4	200	24	24 ± 0.2	51 ± 1.1
5	200	24	23 ± 0.3	61 ± 1.3
6	200	72	18 ± 0.1	66 ± 1.5
7	500	48	18 ± 0.3	19 ± 0.9
8	500	48	17 ± 0.4	19 ± 1.1
9	500	48	20 ± 0.3	19 ± 1.1
10	500	48	19 ± 0.2	16 ± 0.6
11	800	24	18 ± 0.2	19 ± 1.2
12	800	24	18 ± 0.1	21 ± 1.1
13	800	72	16± 0.2	30 ± 1.4
14	800	72	15 ± 0.1	24 ± 0.7
15	800	72	15 ± 0.2	26 ± 0.4
16	800	24	19 ± 0.3	19 ± 0.8

Table 3: Analysis of Variance (ANOVA) for both Myrrh and Thyme

	Myrrh		Thyme	
	F-Value	p-Value	F-Value	p-Value
Model	39.68	< 0.0001	249.81	< 0.0001
A	39.68	< 0.0001	472.73	< 0.0001
B	39.68	< 0.0001	26.89	0.0002
Lack of Fit	3.85	0.0755	2.02	0.1829

A: concentration of (µg/mL); B: incubation time (hours)

Table 4: Validation of the experimental design

Essential oil	Incubation time (hours)	Concentration EO (µg/mL)	of Predicted cell viability (%)	Actual cell viability (%)
Myrrh	72	800	14.75	15 ± 0.1
Thyme	24	800	18.25	19 ± 0.8

The experimental design generated by the Design-Expert software provided optimum values for incubation time and EO concentration to achieve the lowest percentage of cell viability in the treatment of lung cancer cells. Table 4 presents Myrrh's and Thyme's suggested parameter values. For Myrrh, the actual cell viability value was 15%, closely matching the predicted value of 14.75%, resulting in a difference of 0.25%. Similarly, for Thyme, the actual cell viability was 19%, with a predicted value of 18.25%, yielding a difference of 0.75%. Due to the fact that the difference between the 2 EOs' numbers is only <1, it is regarded to be significant. When comparing the 2 EOs, the percentage value for Myrrh is notably lower than that of Thyme. Myrrh inhibited cell growth by approximately 85%, while Thyme inhibited around 81%. This indicates a substantial inhibitory effect of Myrrh on lung cancer cell viability compared to Thyme in the experimental conditions.

3.3 Cytotoxicity of essential oils

After 24 hours of incubation, the Myrrh was introduced to the cells as a treatment once the lung cancer cell had reached a steady condition. The treated cells began to demonstrate the effect, and their numbers started to decline immediately after 24 hours, which is when the exponential phase of the cell cycle takes place. During this phase, cells that were either untreated or treated had reached their maximum growth between 80 and 88 hours. According to the findings, Myrrh successfully inhibited cell proliferation as the number of cell generations was reduced from 4.358 to 3.954.

The process has entered the stationary phase when no growth in the cell concentration exists. When the population is in the stationary phase, the rate of death will be equivalent to the rate of increase. At this stage, the proliferation of cells is restricted

either because there is an insufficient supply of nutrients at a level that cannot support further cell growth or because of an accumulation of metabolic by-products that may have reached a level that is inhibitory to the growth of cells. When the A549 cells were allowed to grow untreated, they reached the maximum cell volume of 3.075 x 10^6 cells/mL. However, when the cells were treated with Myrrh, the maximum cell volume only survived at 2.325 x 10^6 cells/mL.

According to the findings, the growth rate fell at the exponential phase from 0.107 h^{-1} to 0.106 h^{-1}. Even though Myrrh only slowed down growth rates by a modest number, the kinetics analysis conducted as part of this investigation demonstrated that Myrrh possesses inhibitory properties. The majority of chemotherapeutic medications work by interfering with the processes of cell division, making them particularly effective against tumours that are growing. From this experiment, this can be demonstrated as proof when the doubling time (td) is increased from 2.81 to 2.84 hours. When the normal proliferation of cancer cells is interrupted by therapeutic interference, a compelling therapeutic agent can lower the cancer's growth rate. This is the summarised concept of doubling time. This is proven by inspecting any capable therapeutic agent drives to boost the doubling time of cancer cells. In essence, the doubling time serves as a measurable indicator of the impact of a therapeutic intervention on the growth dynamics of cancer cells, highlighting the potential of effective treatments to slow down the rate of cancer cell proliferation.

The final phase of cell growth, known as the death phase, happens naturally due to cell death. During this phase, the viability of cells is at its lowest point. The findings demonstrated that Myrrh caused an increase in the rate of cell death in A549 cells. Compared to untreated cells, which had a death rate of only 0.016 h^{-1}, the death rate was boosted to 0.022 h^{-1}. Drugs that usually cause necrosis in the growing cell can sometimes cause the natural death of cells to be halted or sped up. Necrosis is a non-active process typically occurring due to sudden and extreme stress on cells. According to (Boise & Collins, 2001), this is distinguished by a breakdown of the plasma membrane, which causes the cell to enlarge, ultimately leading to the rupture of the cell.

4. Conclusion

By investigating the potential of EOs as a halal alternative therapeutic source for lung cancer, we have uncovered important findings that advance our understanding that EOs can inhibit the growth of lung cancer. The key findings of this study include the anticancer properties of EOs. The main goal of this study is to prove that EO possesses anticancer abilities by experimenting to check their cytotoxicity effect on lung cancer. All of the EOs in this study inhibited the A549 lung cancer cells. Frankincense and Thyme showed the lowest percentage of cell viability, 6.1% for Frankincense and 6.2% for Thyme. Myrrh and Thyme showed the lowest $IC_{50,}$ possessing cytotoxic activities toward the A549 cell line. Myrrh had the lowest IC_{50} value, 19 µg/mL, followed by Thyme, 45 µg/mL. Based on this optimisation study, Myrrh showed a very good ability to inhibit lung cancer compared to Thyme. The consistency and the percentage number of inhibitions for Myrrh are better than those for Thyme. The design of the experiment (Design-Expert) showed that both the factors, incubation time and concentration of EOs, are important for the cytotoxicity study of cancer cell treatment. A growth kinetics study of A549 cells treated with Myrrh showed reduced cell generation numbers. Even though Myrrh was only responsible for a modest reduction in growth rate and an increment in death rate, the treatment shows the effects, which indicates that Myrrh possesses anticancer activity at least partially through inhibition of cell growth.

Products derived from plants are gaining recognition as promising choices for halal products. Essential oil, extracted from plants and known for their historical use in traditional medicine, has piqued the interest of experts. This research suggests that EO can successfully treat various ailments, including certain types of cancer, such as lung cancer. Essential oil not only offers a halal alternative to pharmaceutical items but also provides a complementary option to chemical products that may have potential negative impacts on consumers. However, it is crucial to conduct further research, including clinical trials, to enhance the efficacy of these treatments and ensure their safety and effectiveness in diverse populations.

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