



Effects of Thyme Essential Oil Chemotypes on Breast and Cervical Cancer Cell Lineages

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Abstract

Purpose: Breast cancer affects 122.2 women per 100,000, while cervical cancer affects 7.4 per 100,000 women in the USA. Research reports that certain thyme essential oils inhibit growth and potentially cause apoptosis of many cancer cell types. A chemotype is a chemically distinct entity within a plant species; *Thymus vulgaris* has 7 possible chemotypes, each of which will change the properties of the essential oil if it is the dominant component. The objective of this study is to determine if thyme essential oils containing different chemotypes have apoptotic activity against MCF7 breast cancer cells and HeLa cervical cancer cells.

Methods: This study has been submitted to the Institutional Review Board for approval. *In vitro* cancer cells will be cultured and maintained using standard ATCC protocols and plated in 96-well plates. Serial dilutions of thyme essential oils (thymol, thujanol, and linalool) will be applied to cells and cultured for 24 hours. Cell viability will be measured using AlamarBlue which undergoes reduction by viable cells resulting in a red fluorescence proportional to the number of living cells. Cancer cell apoptosis will be evaluated using Apostat, a fluorescent caspase inhibitor. Experiments will be performed in triplicate on each cell line (MCF7 and HeLa). Data will be reported as the average percent of viability or average percent of apoptosis compared to the control plus/minus standard error of the mean.

Introduction

Essential oils are extremely concentrated extractions from plants with a distinctive scent, or “essence”. Although these oils have beautiful aromas, they have many uses beyond their scent [1]. Essential oils have been used for the relief of pain, anti-bacterial, and anti-inflammatory properties for thousands of years. Some were so well known for their ability to heal that they were more valuable than gold. Thyme essential oils are extracted from the *Thymus vulgaris* plant and have been identified to have possessed antioxidant properties [2]. Previous research at Sullivan University College of Pharmacy found that 3 thyme chemotypes (thymol, thujanol, and linalool) had apoptotic activity against A549 lung cancer cells. Research regarding the inability to induce apoptosis in breast and cervical cancer cell lines is lacking. Both breast and cervical cancer have a huge impact on the

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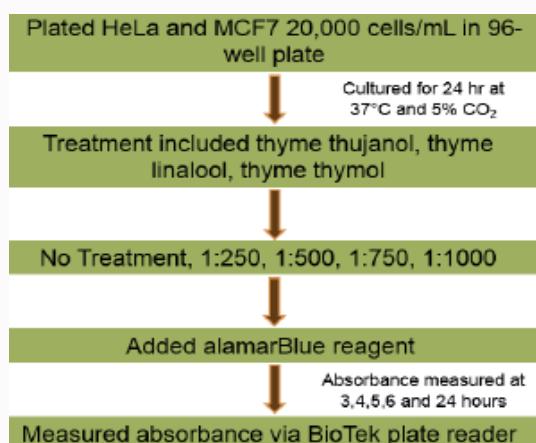


Figure 1: HPLC (High Pressure Liquid Chromatography). HPLC was used as a way to visually represent the similarity of key constituent absorbance levels between chosen essential oils.

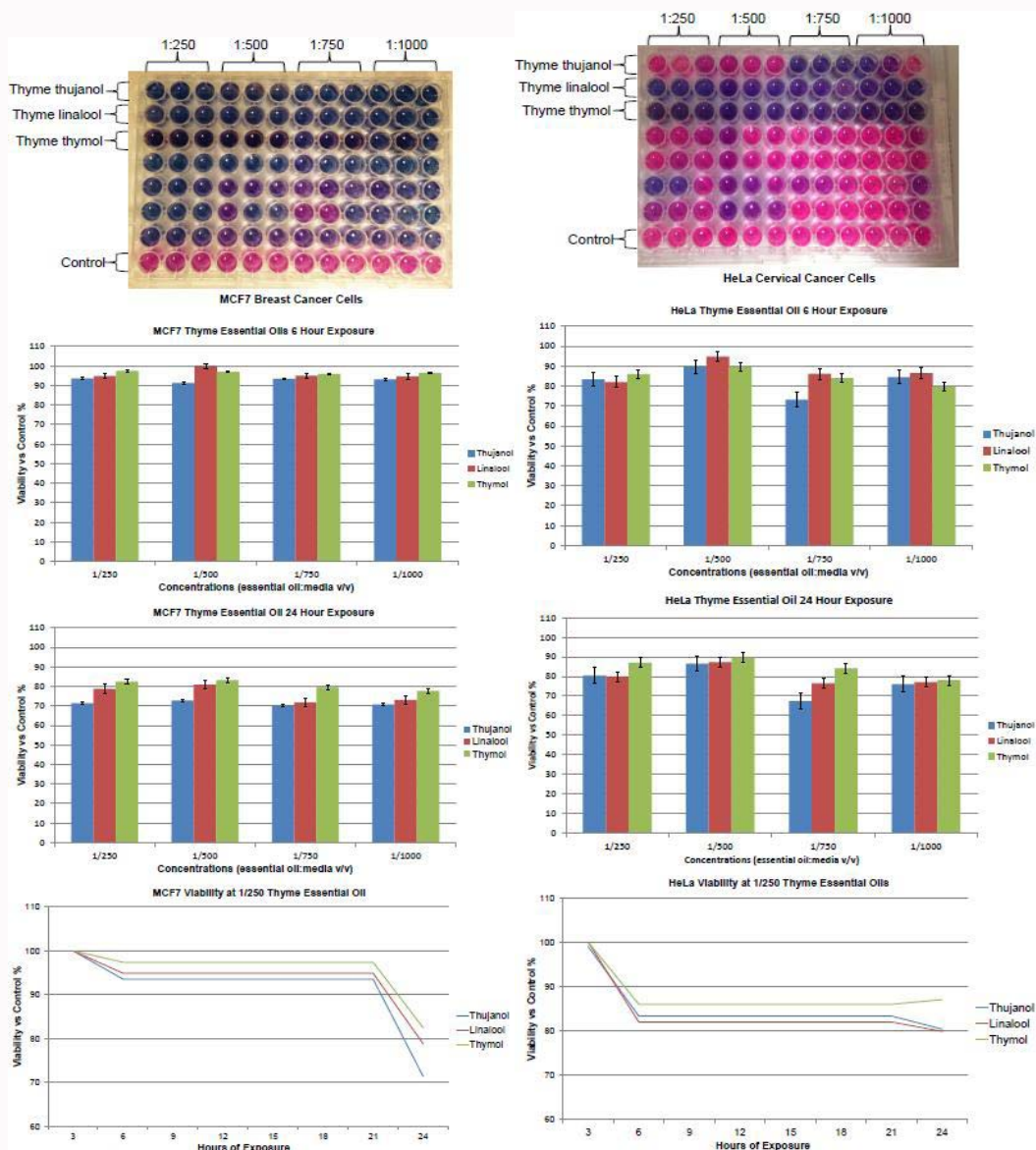


Figure 2: High Pressure Liquid Chromatography Results.

American population. In 2012 24, 145 women and 2, 125 men were diagnosed with breast cancer, while 12, 042 women were diagnosed with cervical cancer. Our hypothesis is that thyme thymol, thyme thujanol, and thyme linalool will have apoptotic activity against HeLa cervical cancer and MCF7 breast cancer cells.

Objectives

Essential oil use has been on the rise over the past decade. Evidence proves that essential oils do have many beneficial properties, including antineoplastic activity. Previous research has shown that thyme essential oils inhibit growth and potentially cause apoptosis of A549 lung cancer cells. *Thymus vulgaris* is a plant species that can have 7 different chemotypes; a chemotype is a chemically distinct entity within a plant species. The objective of this study is to determine if thyme essential oils containing different chemotypes (thujanol, linalool and thymol), have apoptotic activity against MCF7 breast cancer and HeLa cervical cancer cells. If apoptosis is detected, this research could be used to develop novel mechanisms of action for treating breast and/or cervical cancer.

Methods

MCF7 breast cancer cells and HeLa cervical cancer cells were cultured and maintained using standard ATCC protocols. The media used to culture HeLa cells was created with Eagle’s Minimum Essential Medium (EMEM), 10% heat-inactivated fetal bovine serum (FBS), and 1% penicillin-streptomycin. The media used to culture MCF7 was created with EMEM, 10% heat-inactivated FBS, 1% penicillin-streptomycin, and 0.625 mL of 0.01 mg/mL insulin.

Alamar Blue (Cell Viability Detection)

AlamarBlue is a non-toxic reagent used to determine cell viability. The active ingredient, resazurin, is a blue, non-fluorescent, cell-permeable indicator. Resazurin is reduced to resorufin by viable cells, which results in a fluorescence that is red in color. The amount of fluorescence produced is proportional to the number of living cells (Figure 1).

Alamar Blue Results

Based on the results of this study, essential oils of thymol, thujanol,

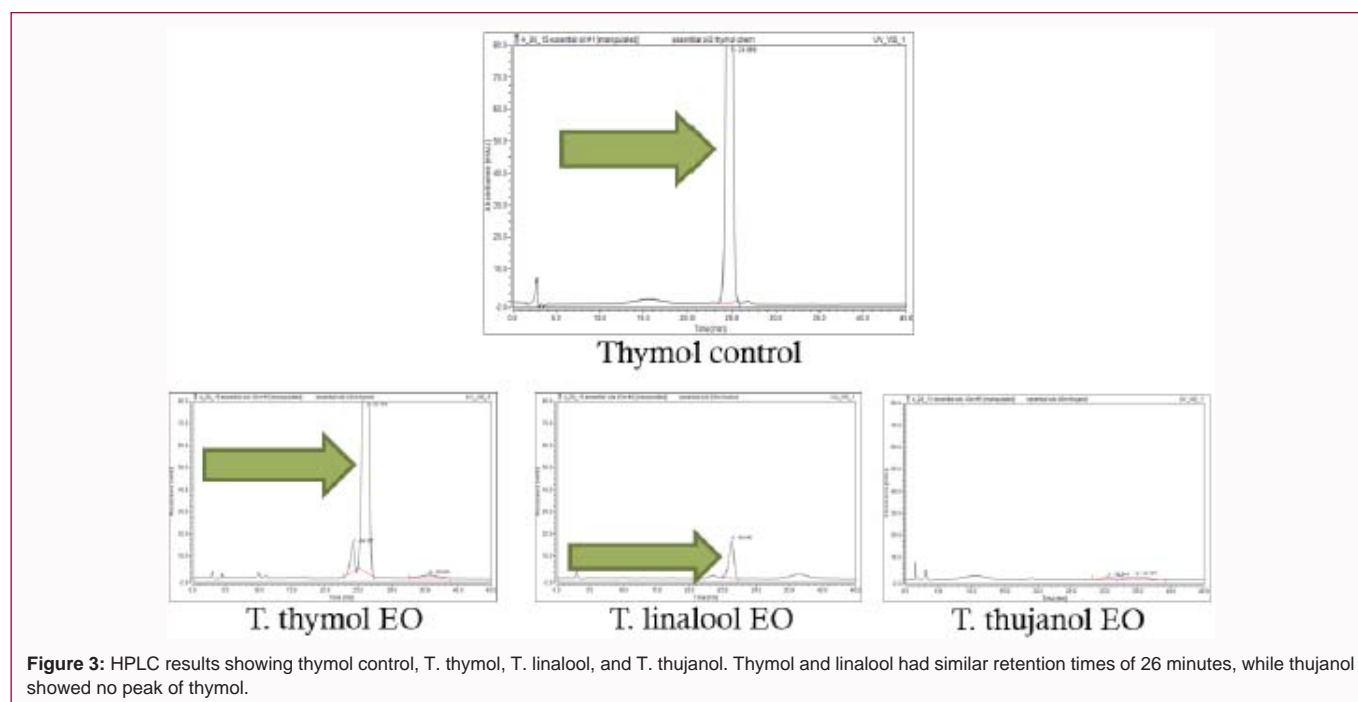


Figure 3: HPLC results showing thymol control, T. thymol, T. linalool, and T. thujanol. Thymol and linalool had similar retention times of 26 minutes, while thujanol showed no peak of thymol.

or linalool decrease HeLa cervical cancer and MCF7 breast cancer cell viability by approximately 20% after 24 hours [3]. Similar induction of apoptosis was seen at all concentrations for both MCF7 and HeLa cells, therefore a dose-dependent relationship was not observed [4] (Figure 2 and 3).

Discussion

Alamar Blue

Thyme thymol, thyme linalool, and thyme thujanol all induce apoptosis of MCF7 and HeLa cells. After 24 hours a 20% decrease in cell viability was observed regardless of the concentration ratio of the essential oils used, with $p < 0.05$ for all essential oils in both cell types. This indicates that at 24 hours there is not a dose-dependent relationship for induction of apoptosis in HeLa and MCF7 cancer cells.

HPLC

Thyme thymol and thyme linalool showed similar retention times to the thymol control, indicating that these essential oils contain thymol. Thyme thujanol did not have the same peak as the control, indicating that there was no thymol present. These results coupled with the alamarBlue results conclude that thymol containing and non-thymol containing thyme essential oils induce apoptosis in HeLa and MCF7 cancer cells.

Limitations and Future Studies

Our study only looked at cell viability after 24 hours, and further studies evaluating cell viability beyond 24 hours could reveal more information regarding the relationship between dose and apoptosis, or whether there is a superior response to one of the essential oils. We were unable to measure absorbance between the 6th and 24th hour of exposure due to the laboratory being closed, which could explain why the line graphs of cell viability vs hour of exposure are so flat. To determine the clinical utility of these essential oils we would need to

evaluate the effect that these concentrations have on healthy human cells. Lastly, further research investigating the mechanism through which these essential oils are inducing apoptosis could lead to the development of novel antineoplastic medications.

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Disclosure

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