Use of a breast cancer model to examine cytotoxicity of common topically applied essential oils

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Abstract

Chemotherapy is a typical form of breast cancer treatment that not only acts on tumor cells, but also has negative systemic effects on non-cancer cells. Natural plant products and compounds derived from plants, such as essential oils, are being researched as alternative forms of cancer treatment due to their anticancer properties. We tested the cytotoxicity of essential oils of bergamot (*Citrus bergamia*), clary sage (*Salvia sclarea*), frankincense (*Boswellia serrata*), lavender (*Lavandula angustifolia*), lemongrass (*Cymbopogon flexuosus*), peppermint (*Mentha piperita*), and tea tree (*Melaleuca alternifolia*) to MCF-7 breast cancer cells at varying concentrations. MTT assay analysis was carried out following treatment to determine cell viability. During treatment, it was observed that there were interactions between the essential oils, due to their volatile nature. The essential oils were then individually tested to determine cytotoxicity to treated and non-treated cells. Cell viability was observed to be the lowest in 0.1% treatment groups. Cells in adjacent, non-treated wells also experienced reduced viability compared to those farthest from the row receiving treatment.

Introduction

Cancers, in general, are characterized by the dysregulation of cell signaling pathways and result in uncontrollable cellular division and proliferation (Amin, R., et al. 2009). Breast cancer is one of the most commonly-diagnosed cancers among women in the United States and is also the second leading cause of cancer-related death among women (Desantis, et al. 2013). Women with primary invasive breast cancer receive local and systemic treatment in order to reduce mortality from breast cancer. Chemotherapy and hormone therapy are two forms of systemic treatment that are given after local treatment (Shapiro and Recht 2001). While chemotherapy is a typical form of breast cancer treatment, it not only acts on tumor cells, but also has toxic effects on non-cancer cells (Yuan, et al. 2016). Due to the adverse side effects, alternative forms of cancer treatment are being explored (Guatam, et al. 2014). Natural plant products and compounds derived from plants, also known as "phytochemicals," are being researched for possible anticancer treatment (Amin, A., et al. 2009; Guatam, et al. 2014). Essential oils are aromatic, highly volatile, and hydrophobic liquids produced by aromatic plants. Their lipophilic nature allows them to easily cross the membranes of cells (Guatam, et al. 2014; Russo, et al. 2015). Essential oils from aromatic plants have been reported to possess antimicrobial, antioxidant, and anticancer properties (Guatam, et al. 2014; Zu, et al. 2010). We hypothesized that treatment of MCF-7 breast cancer cells with the individual essential oils listed would result in significant cell death *in vitro*, and that higher concentrations would result in lower cell viability.

Materials and Methods

For initial studies, serial dilutions of each essential oil, in dimethyl sulfoxide (DMSO), were prepared as stock solutions with concentrations of 1:1, 1:10, 1:100,1:1000, and 1:10000. Preceding each assay, a 1:100 dilution of each stock oil dilution was prepared in culture media for final concentrations of 0.0001% to 1%. An additional 10% peppermint oil solution, in media, was also made before each assay in initial trials.

MCF-7 human breast cancer cells were maintained in a T-75 flask, using MEM media with 5% calf serum. Once cells reached 90% confluence, the cells were removed from the T-75 flask using trypsinization and transferred to a 96-well plate with 100 µl suspended cells per well. Cells were allowed to adhere to the microplate for approximately 24 hours before treatment with essential oil solution. Media from each well was removed and replaced with 100 µl of control (media or media with 1% DMSO) or essential oil solution. Treatment was carried out for 24 and 48 hours in initial trials, and for 24 hours in secondary trials (secondary trails were conducted with concentrations of 0.01% to 1%) in a humidified incubator at 37ºC with 5% CO_2. After each treatment session, MTT (3-(4,5-dimethylthiazolyl-2)- 2,5-diphenyltetrazolium bromide) assay analysis was carried out by adding 5 µl of 5mg/mL of MTT solution to each well. The formazan crystals were resuspended in 200 µl of DMSO and the plates were analyzed using a Bio-Rad iMark Microplate Absorbance Reader at 570 nm. Statistical analysis was performed using Univariate Analysis of Variance (ANOVA) with SPSS (IBM SPSS Statistics 21, IBM Corp., Aramonk, NY, USA), with $p<0.05$ indicating significant variation from the controls.

Results

Initial studies using lavender, peppermint, lemongrass, and the four other essential oils (data not shown) indicated that the volatile nature of the essential oils may have impacted cell survival in nearby wells. We conducted further studies on the essential oils to observe how treated and non-treated cells react when only exposed to one essential oil for 24 hours (as opposed to all of the essential oils being tested on one plate). Lavender, peppermint, and lemongrass were the essential oils that demonstrated the lowest cell viability upon observation, and thus were the first chosen for further analysis.

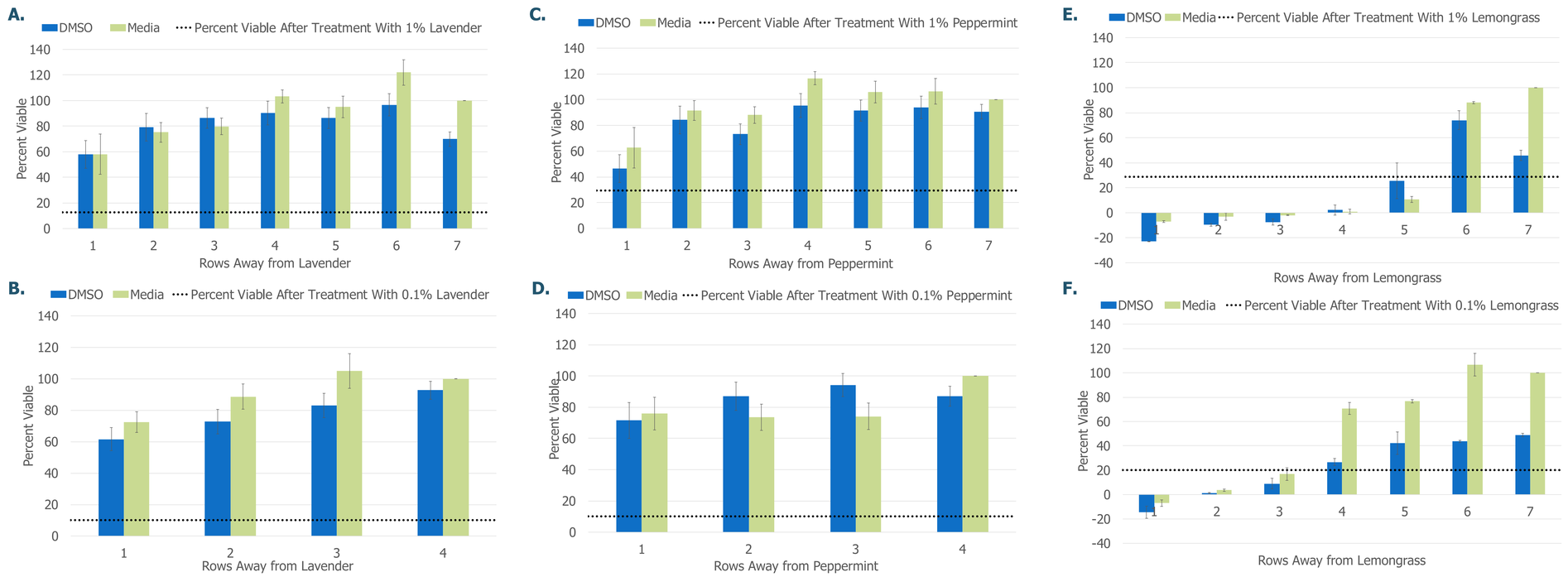

Fig 1. Cell viability following 24-hour treatment with essential oils. Cell viability in oil-treated wells is represented by the dotted line in each graph. Viability in adjacent, untreated wells (with media or DMSO control) 1-7 rows away from the oil treatment is shown. The farthest wells receiving media only were set as a 100% viable standard. In graphs B and D, row 4 was set as the 100% viable standard as there were no statistically significant differences in cell viability between wells 4-7 for these trials. Cell viability after treatment with 1% lavender (A) was reduced to around 12.68%. Cell viability after treatment with 0.1% lavender (B) was reduced to around 10.08%. Cell viability after treatment with 1% peppermint (C) was reduced to around 29.40%. Cell viability after treatment with 0.1% peppermint (D) was reduced to around 10.13%. Cell viability after treatment with 1% lemongrass (E) was reduced to around 28.72%. Cell viability after treatment with 0.1% lemongrass (F) was reduced to around 20.15%.

Conclusions

Cell death was observed in initial trials of cells treated with the essential oils and their varying concentrations, with higher concentrations generally having lower levels of viable cells. However, as subsequent trials were conducted, it was discovered that, due to the volatile nature of the essential oils, cells in adjacent, non-treated wells were also dying. We began separating the essential oils onto individual plates to determine cell viability in treated wells, as well as non-treated wells across the plate. Unfortunately, due to events related to COVID-19, only lavender, peppermint, and lemongrass essential oils were completed using this method. Our hypothesis was partially supported as all oils caused significant cell death in treatment rows, however, treatment with 0.1% essential oil caused more cell death than treatment with 1% essential oil. Cells in adjacent non-treated wells also experienced significant reduced viability compared to those farthest from the row receiving treatment. Thus, nearby cells not directly treated with the oil are still experiencing cytotoxic effects to some extent. Results obtained from treated cells are supported by previous research that suggests that these essential oils suppress cell viability and proliferation, as well as induce apoptosis (Chaouki, et al. 2009; Seyedeh, et al. 2014; Sharma, et al. 2009; Tayarani-Najaran, et al. 2014; Zhao, et al. 2016). Further research would include testing the volatile and cytotoxic nature of the remaining essential oils as well as the mechanisms of cytotoxicity of each individual oil.

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Acknowledgments

We would like to thank the Millikin Biology Department for funding this project as well as providing laboratory space and equipment to work with. We would also like to thank the Leighty Science Scholarship Program for allowing us to get a head start on this project.