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 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.

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. Proceeding each assay, a 1:100 dilution of each stock oil solution 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 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 trials were conducted with concentrations of 0.01% to 1%) in a humidified incubator at 37°C with 5% CO₂. After each treatment session, MTT (3-(4,5-dimethylthiazol-2-yl)-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 Microplate Absorbance Reader at 570 nm. Statistical analysis was performed using Univariate Analysis of Variance (ANOVA) with SPSS (IBM SPSS Statistics 21, IBM Corp., Armonk, NY, USA), with p < 0.05 indicating significant variation from the controls.

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 into two groups. Cells in treated wells were also dying. We began separating the essential oils onto two groups. 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.

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References


