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Cytotoxicity of Essential Oils on Breast Cancer Cell Lines

Shaylee Danielle Green

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by

Shaylee Danielle Green

An Honors Capstone
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Contents

Abstract

Research to find treatments leading to elimination of cells with cancerous properties but with minimal side effects for the patients. Over the years, cancer mortality percentages have decreased as a result of the ongoing research in the field. Natural compounds have historically been effective in eliminating cancer cells, but current treatments still cause a variety of unwanted side-effects even when successful. Essential oils derived from the species *Helichrysum italicum, Juniperus communis, Commiphora myrrha*, and *Picea mariana* showed cytotoxic effects on *in vitro* breast cancer cell lines MCF-7 and HS-578T and could be sources for new anticancer drugs

Introduction

According to the latest cancer statistics by the Center for Disease Control, or CDC, in 2015, cancer was the second leading cause of death in the United States ("USCS Data Visualizations", 2018). According to the study, 1.6 million were diagnosed with cancer in 2015. Of these newly diagnosed cancer cases, breast cancer was the overall number one diagnosed cancer out of all types of cancer.

In the study, the CDC still accounted for 595,919 people dying from a cancer diagnosis. However, statistics from the National Cancer Institute at the National Institutes of Health indicate that this is a 26% decrease in mortality rate from 1991 ("Cancer Statistics", 2018). The American Cancer Society accredits this decrease in cancer mortality rates to research focused on advancing early detection as well as treatment options ("Facts & Figures 2018: Rate of Deaths From Cancer Continues Decline", 2018).

The research goals of this experiment were to find more potential treatment options using natural compounds specifically focusing on breast cancer cell lines. The experimental approach

was to determine the cytotoxicity effects of a variety of essential oils on MCF-7 and HS 578-T breast cancer cells grown in cell culture.

What is cancer?

According to Alberts et al. (2014) in the fourth edition of Essential Cell Biology, cancer is a disease of tissue renewal. There are over 277 different types of cancer, but each cancer requires a number of gene mutations to occur within normal cells before the cancer develops (Hassanpour and Dehghani, 2017). Due to the large variety within cancer types, this section will focus on the basic characteristics of all cancers in order to get a broad understanding.

Cancer progresses by mutations in cells that lead to vital genes no longer functioning properly, which causes the normal cell metabolism and growth pathways to be altered (Hassanpour and Dehghani, 2017). Genetic mutations that lead to cancer typically affect the proto-oncogenes and tumor suppressor genes (Hassanpour and Dehghani, 2017). The genetic changes that occur, such as reducing DNA methylation, altering histones, and affecting nucleosome positioning, affect proto-oncogenes and tumor suppressors which lead to an increase in cell growth and division causing tumors as a result of uncontrolled cellular division. (Hassanpour and Dehghani, 2017).

Tumors can be classified as either benign or malignant. Benign tumors are relatively harmless in most cases since they are non-invasive, meaning they stay localized to the area of presentation, but they still have the potential to cause issues within their area of localization. However, malignant tumors are often more harmful and worrisome since they are invasive, meaning they can, and will without treatment, spread to and affect other areas of the body besides the origination of the abnormal proliferation. Malignant cancer cells are physically

characterized by their large, irregular nucleus, low cytoplasm concentration with the cell containing mostly organelles, including large vacuoles, and alterations in the basal membrane (Baba and Catoi, 2007).

With benign tumors, treatment may or may not be needed depending on where the tumor is located and any problems that are arising as a result of the tumor. If a benign tumor does need treatment, it most often is removed by a surgical procedure. On the other hand, since malignant tumors spread to other regions of the body, the treatments for them are more complex, causing the treatment of malignant tumors to be the basis of most of the present-day cancer treatment research. The malignant tumors are more difficult to treat because of the subpopulations that develop as a result of an original malignant tumor. These subpopulations can vary, from each other and from the original tumor, in terms of aggressiveness, invasiveness, and malignancy (Baba and Catoi, 2007). The research in treatment of malignant cancers typically focuses on finding ways to induce apoptosis and stopping cancer cell growth without causing apoptosis and halting cell growth in other types of cells (Baba and Catoi, 2007).

Natural Compounds Treating Cancer

Seventy-five percent of cancer treating drugs are derived from natural compounds that originate in plants (Mitra and Dash, 2018). These natural compounds work by stimulating or inhibiting pathways that prevent or cause cancer respectively (Mitra and Dash, 2018). This is a very active area of research because standard treatment methods such as chemotherapy and radiation therapy often have significant negative side effects. However, when the natural compounds are used along with other treatment methods, the negative side effects can often be

counteracted, and the natural compounds can also work to kill cancer cells (Mitra and Dash, 2018).

The chemicals that are found within natural cancer-killing drugs affect the cancer cells at specific pathways. Examples of the pathways that are affected are the pathways that lead to apoptosis specifically and gene expression changes that can stop cancer progression (Mitra and Dash, 2018).

Anticancer characteristics have been discovered in chemicals found in everyday foods such as broccoli, carrots, tomatoes, cauliflower, cabbage, green tea, and grapes (Mitra and Dash, 2018). This is from a single study that found anticancer characteristics in some of the chemicals of these common plants. It is important to note that many of these studies are being done in research labs even today.

When a natural compound is found to have anticancer properties, it is then important to purify the compound and identify the active material. The original plant extract is prepared, and it is then tested for anticancer properties using cell cultures. Once specific extracts test positive for anticancer properties on cultured cells, the mixture can be further fractionated and tested. Ideally, a pure substance with cytotoxic activity will be obtained and identified.

Materials and Methods

Cytotoxicity testing of the extracts requires maintaining cell cultures to be tested, as well as performing the actual cytotoxicity testing. The methods for both of these are presented here.

Cell Culture

Growing the cells is the first step to cell culture. There are multiple steps that are completed prior to and during growing cells. The following section describes the different steps in the order completed in the laboratory.

Making Media and Growth Serum

Cells must be grown in specific media that supplies all the nutrients needed and the correct osmolarity for the cells. The stock media solution is made in 1-liter batches, and 100 mL at a time is made into growth serum containing 10% fetal bovine serum.

To make the 1 liter of stock solution, 1 pre-packaged bottle of RPMI-1640 is added to 800 mL of sterile water and thoroughly stirred using a stir bar. Then, in a sterile environment, 26 mL of 7.5% sodium bicarbonate, 15 mL of 1.0 M HEPES, and 10 mL of penicillin-streptomycin (concentration of 10,000 I.U./mL Penicillin, 10,000 µg/mL Streptomycin) is added to the RPMI-1640/water mixture.

Once the mixture is stirred thoroughly again using the stir bar, the pH is adjusted to 7.35 by using a pH meter and NaOH to increase pH as necessary. The mixture volume is then adjusted until the total volume is 1 liter. At this point, it is important to ensure the entire solution is thoroughly stirred one last time.

The mixed solution is now sterilized by filtration through a 0.22 micron filter and transferred to storage bottles using a bottle top filter and vacuum suction in a sterile hood and

environment. From this point on, all cell culture activities take place in a sterile hood with an open flame as well as using 70% alcohol for sterilizing items.

After producing the sterile stock solution, the growth serum is made by taking 90 mL of stock solution and adding 10 mL of fetal bovine serum. The fetal bovine serum is added using an autoclaved pipette that has been flame sterilized. Both the stock media and growth media are stored at 4^{o}C.

Retrieving Cells from storage in liquid nitrogen

To begin a cell culture, the cells must be removed from storage properly. The cells in Dr. Debra Moriarity's lab at The University of Alabama in Huntsville are stored in liquid nitrogen in a Locator 4 Dewar. Once a vial of cells is removed from the liquid nitrogen, they are thawed quickly using a 37^{o}C water bath. Swirling the cells while thawing them helps ensure the cells thaw evenly. The 1.0 ml of cells should thaw within 2-3minutes.

Once the cells are thawed, they are transferred from the 1.5 ml storage tube to a 15 mL conical tube and then 5-10 mL of growth media, that has also been warmed in the water bath, is slowly added to them. The amount of growth media added to the cells depends on what solution they were stored in. If they were stored in DMSO, 5 mL of growth serum is added, and the test tube is centrifuged until there is a cell pellet at the bottom. Once a cell pellet is present, the media is suctioned off, and the cells are resuspended in 10 mL of growth media. However, if the cells were stored in glycerol, 10 mL of growth media is added directly, but still slowly, to the cells. Cells stored in glycerol do not have to be centrifuged out of the freezing medium.

Plating Cells

Once the cells are suspended appropriately in growth media, they are transferred to cell culture flasks. Five mL of the growth serum and cell mixture goes into a T-25 flask. The cells and growth medium should always be pipetted slowly to avoid cell lysis. The cells should also be pipetted to the bottom of the flask to help ensure they adhere on the proper side once they begin growing. The cells are then incubated at 37^{o}C in a humidified incubator with 5% CO_2 atmosphere with the cap of the flask slightly loosened for gas exchange.

Growing and Feeding Cells

Once cells have been successfully plated, they should be observed for growth and fed by media replacement every two days. The observation is done using a phase contrast inverted microscope. Observation is completed by looking to see how much the cells have grown, the density of the growth, and the shape of the cells. Once the cells have been observed, and there are no abnormalities seen within the flask, the cells can be fed.

The cells are fed by removing the growth medium from the flask by suction, being careful to not disturb the side of the flask where cells are adhered. Five mL of fresh growth media is added to each flask, and they are placed back into the incubator with the lids loose.

Passaging (Splitting) Cells

Once cells become greater than 80% confluent within the flask, it is time to split them, or divide them into new flasks.

Before beginning, a trypsin solution in Hanks Balanced Salt Solution (HBSS) with a pH of 7.4 is made using 1 mL of 10X trypsin and 1 mL of HBSS solution, the mixture is then sterile filtered by using a 10 mL luer lock syringe and syringe filter that fits to the syringe. Once the

filter is placed onto the syringe, the mixture is poured into the syringe and transferred to another test tube.

To prepare the cells for splitting, the media is first removed and replaced with 5 mL of HBSS solution in each flask. The HBSS solution can be rocked gently over the cells to wash off any media containing FBS. FBS contains a trypsin inhibitor and needs to be removed before adding the trypsin. Once the cells have been efficiently rinsed with Hanks solution it is removed from the flasks carefully so not to disturb the cells.

The Hanks solution is replaced with the trypsin-HBSS solution made previously. It is added at a volume of 4-5 mL in each flask depending on the amount that came through the filter into the test tube. Once the mixture is added, the flasks are laid flat with the cell-bottom down for one minute at room temperature. Once one minute has passed, the flasks are stood upright. The trypsin-HBSS solution is mostly removed with just enough left to cover the bottom of the flask when laying it cell-side down. Once this amount is left in both flasks, they should be incubated cell-side down for two minutes at 37^{o}C in the incubator. After two minutes in the incubator, 5 mL of growth media is added to each flask. The growth media is pipetted carefully on the cell-growth side on each flask. Pipetting the serum up and down may be necessary to suspend all cells in the growth serum. Once this is complete for both flasks, the cells of both flasks are transferred to a single test tube. During the transfer, any stringy material (DNA from lysed cells) should not be pipetted into the test tube if possible. The transfer should also be done slowly so the cells are not harmed.

Once the transfer to the test tube is complete, the test tube is centrifuged for 2 minutes until a cell pellet is formed at the bottom of the tube. Upon completion of centrifugation, the media is removed from the test tube while being careful to not disturb the cell pellet. The cell

pellet is then resuspended in 10 mL of media slowly by pipetting up and down instead of adding the entire 10 mL at once. Gentle stirring with the pipette tip also helps to resuspend the cell pellet.

The hemocytometer is now used to count the cells to calculate the volume of cells that should be plated for a correct concentration. The following should be placed into a small disposal tube: 100 µL suspended cells, 300 µL 0.4% Trypan Blue, 600 µL of 1% NaCl. The solution is mixed by vortex from sliding a finger along the side of the tube. A small amount is pipetted on to each side of the hemocytometer that has a cover slip placed on top. The amount is added at the indention that can be seen on the hemocytometer. It is important to not put too much of the mixture or too little of the mixture on the hemocytometer. Once the hemocytometer is filled appropriately it can be put on a bright-field microscope and focused within the large squares of one side of the hemocytometer. There are four large squares on each side with more smaller squares inside each large square. The live cells are the clear circles present. The Trypan Blue stains the dead cells blue. The live cells are counted in each large square, and the number is then averaged to get the most accurate count possible. Then, using that average, the following calculations can be completed:

$$\frac{\text{Average \# of cells} * 10^4 \frac{\text{cells}}{\text{mL}}}{\text{Goal \# of cells}} * \text{Dilution Factor}$$

The goal number of cells needed depends on the type of cell and whether the cell will be plated in a flask or in a 96-well plate (needed for cytotoxicity assays). For this experiment, the goal number of total cells for plating in 2 T-25 flasks was $3.5 * 10^5$, and it was $5.75 * 10^5$ for plating in 96-well plates. Once the division is done and the final answer is a number in mL, this is the volume from the trypsinized cell suspension that is used. This volume will be subtracted from the total volume being used (10 mL for 2 T-25 flasks, and 12 mL for each 96-well plate). Growth

media will be used for the remaining volume to reach 10 mL or 12 mL. This new suspension of cells and growth media and cells are put in a new test tube, and the remaining cells that were not used for the new concentration are either frozen for use later or disposed of.

To plate the flasks, the same steps are followed as when plating after taking the cells from storage. That is slowly pipette the 10 mL of cells and growth media and transfer it to two flasks. Each flask gets a volume of 5 mL pipetted on the bottom of the flask, and they are stored with loose lids in an incubator. The rack with flasks is gently shaken to evenly distribute the cells over the surface of the flask.

To plate cells to a 96-well plate, the new concentration of 12 mL is put into a sterile reservoir used with multichannel pipettes. Then, an 8 channel multichannel pipette with wide-bore tips is used to transfer the cell suspension to each column of wells within the 96-well plate. The 96-well plate is then stored in the incubator with the top on.

Cytotoxicity Assays

Once the cells have been plated in a 96-well plate, preparation for the cytotoxicity assay can begin. The 96-well plate should be allowed to undergo cell growth for 48 hours before media is removed and replaced with compounds to be tested.

To prepare the compounds that are to be tested for cytotoxic effects, the compounds are retrieved from Dr. William Setzer in UAH's Chemistry Department. The compounds are then diluted to the desired concentration using media and the compound. These are stored in 1 mL plastic tubes until it is time to add them to the plate. There are 3 controls that are always used during cytotoxicity assays: 4 wells of media for the negative control, 4 wells of tingenone for the

positive control, and 8 wells of DMSO as the solvent control because this is what the crude extracts of essential oils are mixed with.

After 48 hours has passed, the media is removed from the 96-well plate using a micropipette tip on the suction canula. During this, it is important to not touch where the cells are growing. If it must be touched with the tip, it is done on an edge to disturb as few cells as possible that may affect the spectrophotometry reading. The necessary compounds and controls are added to 4 wells at a time in each column (except DMSO is added to 8 to keep the first two rows as controls). Therefore, there will be two different compounds in each column of 8- one compound in the first 4 wells, and another compound in the last 4 wells. Once the compounds have been added, the 96-well plate is replaced in the incubator for another 48 hours.

Once the second 48 hours has passed, the 96-well plate should be observed by microscope to get an idea of which compounds killed the cells and which compounds did not. The compounds are removed the same way media was previously removed, and the compounds are replaced with 100 µL of MTT assay solution. The MTT assay solution is made with 1.2 mL of stock 25 mg/ml MTT and 10.8 mL of growth serum. As soon as this solution is added, the plate is placed in the microplate spectrophotometer, the SpectraMax Plus by Molecular Devices, that is set to wavelength of 570 nm. The plate is then read by the plate reader using the program SoftMax Pro Version 6.5.1. This data is saved as the pre-read to the computer for later use, and the plate is then placed in the 37^{o}C incubator for 3 hours.

Once 3 hours has passed, there should be some obvious and consistent color changes among the wells. The MTT Assay measures cell concentration by the reduction of yellow tetrazolium, present in the MTT stock solution. The reduction is completed by metabolically active, or live cells, which ultimately results in intracellular purple formazan. The absorbance

will thus increase with the number of cells present due to the yellow tetrazolium being reduced resulting in increased purple color. The MTT Assay solution is removed from the compounds the same way media and compounds were removed previously. Iso-PBS is added to the 96-well plate using a micropipette to dissolve the purple formazan crystals formed by the MTT Assay solution. The plate can be put on the vortex with a plate holder to increase the speed of the crystal dissolving.

Once the crystals have been dissolved thoroughly, the plate goes back into the plate reader and the same program is used again to get a post-read. This post-read and the previous obtained pre-read are then placed in an excel spreadsheet with calculations built in that uses the spectrophotometric readings to get a percent kill number as well as a standard deviation of that number. This excel spreadsheet is what is used to get the numbers found in the results. Once the post-read is complete, the 96-well plate can be thrown away.

Results

The tables below show the results of the trials testing the cytotoxicity of a variety of essential oils on both MCF-7 and HS578-T breast cancer cell lines. The highlighting indicates essential oils that showed a percent kill of at least ninety percent during a minimum of one trial, and a percent kill of at least sixty-five percent on a second given trial.

Table I shows that HEITEO, JUCOEO, COMYEO, and PIMAEO all meet guidelines that indicate they are potentially cytotoxic to the MCF-7 breast cancer cell line at a concentration of 0.01%. *Table II* shows results that indicate the same four essential oils are also potentially cytotoxic to the HS578-T breast cancer cell line.

Cytotoxicity of Essential Oils on MCF-7 Cells Through 3 Trials

Oil	% Kill Trial 1	% Kill Trial 2	% Kill Trial 3
SASCEO 0.01%	-41.34	36.83	-5.53
HEITEO 0.01%	106.24	104.57	67.97
JUCOEO 0.01%	106.57	103.42	63.68
COMYEO 0.01%	80.84	110.71	56.17
CIBEEO 0.01%	10.72	95.40	-14.98
PECREO 0.01%	55.05	-8.33	-33.75
ALVIEO 0.01%	31.65	-18.42	-10.68
PIMAEO 0.01%	102.87	105.23	27.48
PEGREO 0.01%	92.13	23.44	3.61
PECRCEO 0.01%	104.81	20.17	4.21
SAOFEO 0.01%	-14.92	15.83	3.77

Table I: The table shows three different trials testing the same essential oils on MCF-7 cells.

Cytotoxicity of Essential Oils on HS578-T Cells Through 2 Trials

Oil	% Kill Trial 1	% Kill Trial 2
SASCEO 0.01%	37.38	5.29
HEITEO 0.01%	68.77	91.61
JUCOEO 0.01%	79.20	96.93
COMYEO 0.01%	137.07	92.95
CIBEEO 0.01%	82.02	-4.09
PECREO 0.01%	15.98	-11.29
ALVIEO 0.01%	101.54	-16.55
PIMAEO 0.01%	94.43	99.18
PEGREO 0.01%	67.90	23.50
PECRCEO 0.01%	-3.94	-8.28
SAOFEO 0.01%	40.62	-17.65

Table II: The table shows two different trials testing the same essential oils on HS578-T cells. These are the same oils that were tested in the previous table on MCF-7 cells.

Discussion

Throughout the assays on both MCF-7 and HS578-T cell lines, there are trends that support the essential oils HEITEO, JUCOEO, COMYEO, and PIMAEO are cytotoxic to both cell lines. These inferences were made by observing the oils that had at least one percent kill rate of greater than ninety percent, and then observing the other percent kill rate of the essential oil to determine if the other(s) percent kill rate(s) was greater than sixty-five percent. The essential oils ALVIEO and CIBEEO show inconsistent results within the HS578-T cell line, and a third trial for these specific essential oils should be performed to determine their cytotoxicity to HS578-T. However, ALVIEO and CIBEEO do not seem to be cytotoxic to the MCF-7 cell line. This is inferred because they do not have at least one percent kill rate greater than ninety percent along with a percent kill rate greater than sixty-five percent.

Three essential oils, CIBEEO, PEGREO, PECRCEO, all showed potential to be cytotoxic in a single trial out of the three trials done with the MCF-7 breast cancer cell line. CIBEEO showed potential cytotoxicity during the second trial, while PEGREO and PECRCEO both showed potential cytotoxicity in Trial 1. Since these showed low percent kills in the two other trials, it is likely there was an experimental error to cause the high percent kill in the single trial. The essential oils could have had too high of a concentration of DMSO, the MCF-7 cells could have been plated at too low of a concentration to grow well, or there could have been a reading error with the plate spectrophotometer.

With the HS578-T breast cancer cell line, CIBEEO, ALVIEO, and PEGREO, all have the potential to indicate cytotoxicity since they meet the guideline of at least sixty-five percent kill in one trial. However, each will need to undergo a third trial to determine whether their given high percent kill rates were a result of potential cytotoxicity or a result of experimental error as in the CIBEEO, PEGREO, and PECRCEO were in the MCF-7 breast cancer cell lines.

Cell Line Differences

Though the two cell lines are different when it comes to receptors that are linked to cancer cells, they show similar results in what is cytotoxic to each one. The differences between the cells lie in the estrogen and progesterone receptors, and both cell lines do not have amplified HER-2 which is a growth factor receptor that is sometimes amplified in cancer cells (Kenny et al., 2007).

MCF-7 cells contain both estrogen and progesterone receptors (Kenny et al., 2007). The MCF-7 cells are one of the only cell lines that can maintain their estrogen receptors in cell culture (Lee, Oesterreich, and Davidson, 2015). This feature has caused the cells to be a primary model for the way estrogen receptors will respond to different therapies (Lee, Oesterreich, and Davidson, 2015). This is important because though *in vitro* estrogen receptor-positive cells are harder to come by, they are not as uncommon *in vivo.*

On the contrary, the HS-578T cells do not contain estrogen or progesterone receptors (Chavez, Garimella, and Lipkowitz 2011) or the HER-2 receptor. These cells are referred to as triple negative breast cancer cells due to their lack of receptors. Triple-negative breast cancer constitutes 15-20% of all diagnosed breast cancer (Irvin and Carey, 2008). Chemotherapy is currently the most effective treatment for triple negative breast cancer cells, and there are very few other therapies that have long-term results on this type of cancer (Abramson et al., 2014). Therefore, the goal of researching the natural compounds on this cell-line is in hopes of finding treatments that target the triple-negative HS578-T cells specifically, unlike chemotherapy that kills all dividing cells regardless if they are healthy or unhealthy.

Essential Oils

With the results given, there are 4 essential oils tested that gave results that support the oils are likely cytotoxic to both breast cancer cell lines: HEITEO, JUCOEO, COMYEO, and PIMAEO. These are all abbreviations used in place of the real name of the compound during the research. Dr. William Setzer has created and maintained these abbreviations as necessary. In this section, we will explore the common identities of each of the essential oils as well as their history in medicine.

Essential Oil HEITEO

HEITEO is an abbreviation for *Helichrysum italicum*. Common names for this plant include immortale, sandy everlasting, and curry plant (Klader et al., 2015). The plant is found in the Mediterranean countries in rocky areas that get lots of sunlight (Klader et al., 2015).

In traditional medicine, *Helichrysum* was used on scars and cuts to enhance healing (Klader et al., 2015). This healing property led researchers to study the properties of the plant further. With research, it has been well established that the plant carries anti-inflammatory, antioxidant, and astringent properties (Klader et al., 2015). These likely explain the usage of the plant for cuts and scars since it would likely cause the area to stop swelling and get rid of redness with the anti-inflammatory properties, and it would likely relieve the symptoms of a mild cut by the astringent properties of constricting the skin cells.

Essential Oil JUCOEO

JUCOEO stands for *Juniperus communis.* This plant is commonly referred to as Juniper Berry, Common Juniper, or simply Juniper (Bais et al., 2014). Juniper was also used as

traditional medicine which led to further research of the plant like most of the others that had cytotoxic effects in the experiment. It is a small evergreen tree or shrub containing three main features, and it is found in Europe, South Asia, & North America natively (Bais et al., 2014).

The three different components of Juniper are the bark, the leaves, and the berries. Each different part of the plant has been found to have different effects in medicine. The components have been found to have diuretic, anti-inflammatory, and antiseptic effects (Bais et al., 2014).

Essential Oil COMYEO

COMYEO is the abbreviation used for *Commiphora myrrha* or most commonly known as myrrh. Myrrh comes from an ancient history, and it was commonly used in traditional Chinese and Greek medicine among many (Ahamad et al., 2017). Due to its wide spread use in medicine, the plant has undergone many studies. The Chinese culture currently uses myrrh for treating traumatic injury, inflammation, and tumors (Nomicos, 2007). However, in the United States, the FDA has only approved myrrh as a flavoring substance in food because of possible toxicity effects that can occur at higher levels than is recommended (Nomicos, 2007). These effects include decreased breathing, lethargy, and loss of voluntary muscle movement control, but these effects have only been observed in animals (Nomicos, 2007). Myrrh has been used for effective treatment of lowering cholesterol, advancing wound healing, and parasitic infections in research studies completed in other countries such as Egypt and India (Nomicos, 2007).

The plant is found in the largest amounts in Eastern Africa, Arabia, and India (Ahamad et al. 2017). It exists in extremely hot/tropical environments as a shrub or tree (Nomicos, 2007).

Essential Oil PIMAEO

Picea mariana is the plant that uses abbreviation PIMAEO. The plant is a tree commonly known as the black spruce. The tree is widely known for its high-quality wood in Canadian forests (Francezon, Meda, & Stevanovic, 2017). Contrary to the other essential oils researched thus far, it is not widely known for its medicine purposes. However, extracts from black spruce bark have been found to have antioxidant and anti-inflammatory properties (Garcia-Perez et al., 2012).

Conclusion

Natural compounds in the treatment of cancer is not a new topic. However, there are still naturally existing compounds that have not been used for potential treatment of cancer. Essential oils derived from *Helichrysum italicum Juniperus communis Commiphora myrrha Picea mariana* out of a variety of essential oil compounds, chosen based on known properties and uses of the species, all showed to be cytotoxic on HS-578T and MCF-7 breast cancer cells *in vitro.* The species should undergo further fractioning to discover the pure substance in each species that is causing the cytotoxicity. Once discovered, the substance(s) can be applied to other cancerous and non-cancerous cells *in vitro* to determine their effects before using the cells *in vitro* for potential cancer treatment. The research of cancer treatments by natural compounds should continue to be expanded upon due to the expansive list of compounds that has not been tested.

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