# Possible Roles for Essential Oils in Chemoprevention and Suppression of Cancer

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Abstract

Cancer represents one of the costliest and most prevalent diseases to afflict the modern world, even though treatments have evolved steadily over the years and produce an increasingly positive outlook with each development and innovation. Essential oils have been used medicinally- among other purposes- for thousands of years and have begun to attract attention for possible applications to the field of oncology. Numerous investigations and publications have shed light on the possible chemopreventative (antioxidant and antimetastatic) uses of these oils, alongside cancer suppressive (apoptosis-inducing and cytotoxic) abilities that they may possess. With the high annual incidence of cancer and the ever-rising price of treatment, clinical application of essential oils may transform into a viable and effective compliment to current treatments.

# Possible Roles for Essential Oils in Chemoprevention and Suppression of Cancer

## Introduction

An assortment of claims and assertions have been made regarding the perceived medicinal effects of essential oils (EO), including soothing abilities, alleviating intestinal or headache pains, antidepressant activity, and preventing or even treating various forms of cancer (Barocelli et al., 2004). These claims reach far back into human history, well before the biochemical bases of their abilities could be understood. An example of such a history is found with camphor and its naturally derived oils which were widely used during the time of the Black Death in 14th century Europe for fumigation, as they were believed to have certain illness-preventing effects (though their actual antimicrobial and disinfectant capacities would not be understood for several hundred more years) (Chen, Vermaak, & Viljoen, 2013). EOs have undergone a significant evolution over hundreds of years in terms of their ascribed uses and the available scientific understanding of their mechanisms of actions. Many of the claims concerning these products have not been substantiated scientifically, prompting opposition from regulatory agencies such as the FDA to their use in traditional medical applications (Mitchell, 2014). Nonetheless, while transformed in nature with time, EOs remain a common topic of discussion in the realm of holistic and natural approaches to medicine.

Whereas the aromatherapeutic and antimicrobial effects of numerous different EOs have been topics of study for many years, investigation into EOs as a supplementary approach to cancer prevention and treatment is a topic of high interest which has emerged with the growth in understanding of cancer biology in the modern era. When taking into account the immense cost of cancer care and the large demographic afflicted by the disease, any possible amelioration to the current state of treatment and prevention are valuable areas of investigation.

This literature will seek to address the biochemical basis of the roles EOs may play in cancer prevention and treatment and provide an assessment of the current state of knowledge concerning their contribution to oncology as supplementary treatment options. With considerable research performed in recent years regarding oncological applications of natural products, EOs merit an appropriate assessment of their broad-scope application to the field of oncology.

## Definition and History Essential Oils

### The Nature of Essential Oils

To properly understand EOs in the context of oncological applications, it is important to first understand the origins and nature of these oils. Much mysticism has existed surrounding EOs, including their extraction and purification processes, their chemical makeups, and their mechanisms of action.

Today, EOs are defined as the product of some form of distillation (be it hydrodistillation, steam distillation, or some alternative form) or a mechanical process that causes the tissue matrices to release their volatile fractions- a mixture of easily vaporizable compounds under suitable conditions (Alzamora et al., 2010). Under this definition, the makeup of EOs cannot be defined as variations of a single chemical compound, but rather as mixtures of a variety of compounds that vaporize readily under favorable conditions. Commonly comprised of lipophilic plant metabolites, the identities of the chemical components of EOs vary widely between oils, but generally have similar chemical characteristics and molecular weights (typically under 300 amu) (Turek & Stinting, 2013). In fact, the number of compounds that comprise a given oil may range from just a few to in the hundreds, with their relative concentrations determining the characteristic smells, tastes and other qualities of each oil; these characteristics are known to even vary from plant to plant depending on the growth conditions of

the given plant and the extraction process employed (Turek & Stinting, 2013). For example, two peppermint plants grown in different regions may both yield peppermint EO, though the conditions under which the plants grew and the manner in which the peppermint oil was isolated may produce two oils that vary noticeably in their relative concentrations of their constituents.

Among chemical constituents a wide range of chemical classes are represented, including ketones, aldehydes, alcohols, phenols, oxides, esters and a variety of unsaturated hydrocarbons (Turek & Stinting, 2013). Many EOs are found to contain terpenoids (unsaturated derivatives of terpene containing a number of linked isoprene subunits), which include such compounds in their mono-, bi-, tricyclic mono-, and sesquiterpenoid forms (Turek & Stinting, 2013). Diterpenes are relatively low in concentration in most EOs due to the volatility arising from their relatively low molecular weights which causes their loss during most distillation procedures (Turek & Stinting, 2013). Nonetheless, diterpenes still represent a significant component in a large number of oils. Aliphatic derivatives of short hydrocarbons are often seen in significant quantities in EOs, though they contribute to the oils' characteristics in a lesser manner than terpenoids (Turek & Stinting, 2013). Due to their hydrophobic structures, many oil constituents are low in aqueous solubility, and thus must form emulsions via interaction with surfactants (Boire, Reidel & Parrish, 2013).

**History of Essential Oils**

The dawn of production and use of EOs extends as far into history as Ancient Egypt around 4500 BC where they were commonly employed in herbal medicine and preparing cosmetics (Elshafie & Camele, 2017). Records indicate the incorporation of EO into medical practices of Ancient China and India between the years of 3000 and 2000 BC- a time during which a list of over seven hundred oils were considered to be viable medicines (Elshafie &

Camele, 2017). The Greeks did not begin to employ EOs until about 400 BC, though their oils represented those from a new population of plants found in the Mediterranean, further expanding the origins from which these oils were extracted (Elshafie & Camele, 2017). EOs were deemed so valuable in their applications ranging from combatting inflammation to treating infection that ancient Hebrews called myrrh "holy oil" and considered it of greater monetary worth than gold (Boire, Reidel & Parrish, 2013). Ancient Egyptian priests buried King Tutankhamun with 350 liters of aromatic oils as a symbol of its worth (Boire, Reidel & Parrish, 2013). Historical texts, including the Bible, make frequent mention of aromatic oils, serving as a testament to the common application of these compounds for daily medicinal use (Boire, Reidel & Parrish, 2013). Applications at the time often included treatment for joint pain, wounds, skin sores, burns and even larger ailments such as leprosy or sexually transmitted diseases (Boire, Reidel & Parrish, 2013).

In the 19th century, a number of vegetable EOs were included in the Romanian pharmacopoeia, and the Institute of Medicinal Plants was founded in 1904 (Vinatoru et al., 1996). Commercial production and sales of EOs began in the 1940's with world-wide interest and exploitation expanding ever since (Vinatoru et al., 1996). Over the many years of EO production, the methods of extraction have varied greatly, with modern investigation and suggestion of genetically modified plants that yield greater quantities of oil constituents to further improve current production methods (Bertoemu, Sales, Ros, Arrillaga & Segura, 2007). Ancient methods included a technique known as enfleurage, in which plants were combined with solid, odorless purified fats and allowed to absorb the volatile compounds released by the plant (Cortez-Pereira, Baby & Velasco, 2010). With time and the development of technology, this

technique was replaced with alternatives such as various forms of distillation and solvent extraction (Cortez-Pereira, Baby & Velasco, 2010).

As seen with innumerable technologies, medicinal practices, and processes, the production, uses and understandings of EOs have undergone a dramatic transformation over the many years of their exploitation by humankind. Recognition of this evolution serves to highlight the long-standing value that EOs have maintained and shed light on the significance of investigating their ascribed medicinal abilities. Oncological use of these natural products is one such field.

In order to fully understand application of EOs to oncology, it is first important to explore the mechanisms of cancer development and proliferation. In light of the molecular biology of cancer, the relevance of EOs as a possible addition to current treatment methods may be clarified.

## Cancer

The onset, treatment, prevention, and cost of cancer is an extremely prevalent topic in classrooms, laboratories, media, and household conversations alike. Of course, modern technology has brought- and continues to bring- new methods of disease detection and the ability to treat an ever-wider array of types of cancer by employing an ever-growing set of procedures, drugs and therapies. Nonetheless, modern medicine has yet to “cure” cancer, nor provide completely effective treatment or prevention options for the many forms of cancers that may arise in an individual. As such, the incidence of cancer occurrence is still high, even if the outlook of a cancer diagnosis in today’s medical age may be much more desirable than one in years prior. As cancer continues to be a major treatment need globally, expansion is continually needed in current understanding of its molecular biology and means of counteracting its negative

effects on those afflicted. As such, the possibility of using EOs alongside other treatment methods offers another beacon of hope in expansion of current methods to hopefully improve the current outlook of diagnoses and cost of remediating them.

**The Cost of Cancer Care**

The total cost of cancer care is projected to total $173 billion in 2020 in the United States alone (Mariotto, Yabroff, Shao, Feuer & Brown, 2011). Although rapid development in the 1990's in the field of oncology led to a dramatic decrease in cancer incidence and an increase in survivability, the net cost of care also climbed significantly (Mariotto, Yabroff, Shao, Feuer & Brown, 2011).

According to the American Cancer Society, cancer deaths dropped by 22% between 1991 and 2011 (Siegel, Miller & Jemal, 2015). However, an increase of approximately 75% in the overall cost of cancer treatment was also observed in the years 1995 to 2004, believed to be largely attributable to the development of surgical techniques and pharmaceuticals which were effective but increasingly costly; in 2004 this cost accounted for just under 5% of the United States total medical care costs (Mariotto, Yabroff, Shao, Feuer & Brown, 2015). As oncology continues to grow as a discipline, the inverse relationship between cancer deaths and treatment cost continues to grow. Each reduction in deaths attributable to cancer is met with an increase in the per-capita cost of doing so.

The high national cost is understandable considering cancer's place as the second leading cause of death in the United States (and many other countries), with the expectation it will surpass heart disease in the near future to become the leading cause of death (Siegel, Miller & Jemal, 2015). As a result, the dialogue surrounding cancer care warrants expansion beyond how to improve cancer care and outlook (an indubitably important discussion). In what ways could

improvements continue to be made without drastic increases in cost? What natural methods may be employed to aid treatment? How may one mitigate the negative side effects (such as recoveries from invasive surgeries or the side-effects of chemotherapy) of existing treatments while still providing the highest quality care?

EOs certainly do not offer an answer-all to this predicament. However, should viable implementations of these natural compounds be found to aid in cancer treatment or prevention, they are low-cost, natural, renewable additions to current methods, and may provide an opportunity to improve current care without reducing affordability. As such, this study intends to identify current literature which demonstrates the preventative and treatment capacities which essential oils may offer as a holistic addition to modern approaches to cancer care.

**Molecular Biology of Cancer**

Nevertheless, the field of oncology is extremely complex and the task of preventing or treating cancer is made difficult by the various mechanisms which cause cancer to arise and proliferate. In a broad sense, cancer development is rooted in genetic mutations which foster uncontrolled cell growth and tumor propagation (Fenech, 2002). However, to stop at this definition is an oversimplification of the intricate molecular biology dictating cancer. A comprehensive examination of the underlying causes of cancer's onset and progression is beyond the scope of this literature, though a number of key portions are essential in understanding the application of EOs to oncology.

Normal physiology of somatic cells is tightly regulated in terms of cell's regulation through regular cycles of growth and division, dictated differently in tissues throughout the body. However, a number of factors may afflict this careful regulation and cause cells to exist in a state of deregulated growth and division (Fenech, 2002). Altered gene expression may be caused by

events such as gene mutation, rearrangement of chromosomes, and chromosomal nondisjunction, which cause one or more of the genes responsible for regulation to be affected (Fenech, 2002). As a result, expression of a given protein involved in the normal cell cycle may be increased or decreased such that the cell begins to divide rapidly, proliferate abnormally, and disrupt the normal physiology of the tissue, organ, and organism (Fenech, 2002). Proteins such as p53, which suppresses tumor formation and slows growth, may be decreased in expression, whereas proteins such as certain transcription factors and protein kinases may be upregulated and support uncontrolled growth (Li, Sun & Wang, 2017).

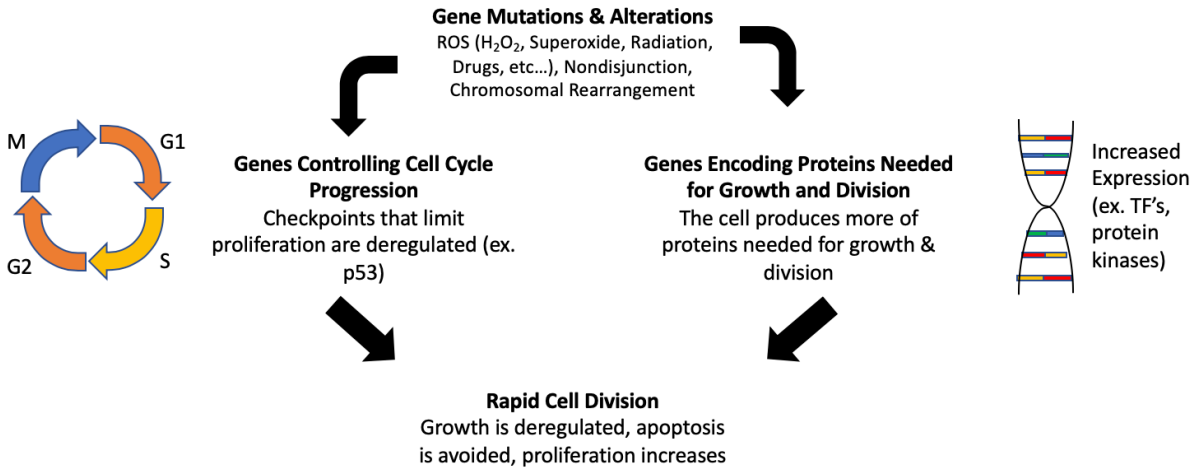


*Figure 1. Changes in gene expression allow uncontrolled cell growth.* Genetic mutations and alterations arise from a number of sources (mutations, chromosomal nondisjunction or rearrangement, strand breakage, etc…) which affect their expression and ability to accurately code for their protein. Proteins responsible for tight regulation of progression through the cell cycle are insufficiently produced, causing dysregulation of the cycle, permitting rapid divisions. Genes encoding proteins needed for normal growth and division, such as certain transcription factors or protein kinases, may become over expressed and produced in excess, permitting proliferation.

## Causes of Cancer Onset

Almost all cancers have been shown to exhibit high rates of what are known as reactive oxidative species (ROS), which have subsequently been heavily implicated in tumorigenesis

(Liou & Storz, 2009). ROS induce what is called oxidative stress on the cells in which they are found, in turn causing damage to cellular structures such as DNA, proteins and organelles; damage to DNA often ends with mutations which may vary in severity yet commonly cause carcinogenesis (Kumari et al., 2018). Alongside elevated ROS levels, cancer cells often express high levels of antioxidant capacity as well to survive and prevent ROS activity from causing cell death (Kumari et al., 2018).

ROS arise in a variety of manners. They are a normal yet toxic byproduct of oxidative phosphorylation, which may be amplified when initial electron carriers within the electron transport chain remain reduced (such as in the case of excessive caloric intake), yielding the undesired product of superoxide, $O_2^-$ (Wallace, 2005). Although this ion is converted to hydrogen peroxide in the mitochondrial matrix, it may be further converted into a hydroxyl radical in the cytosol, a highly reactive ROS, which may then induce oxidative stress and damage upon the cell (Wallace, 2005). Mitochondrial superoxide production is believed to be the largest producer of cellular ROS, consuming as much as 1% of the cell's total oxygen intake in the production of superoxide alone (Sullivan & Chandel, 2014). This is one of a number of endogenous sources of these species, which may be extended to include the endoplasmic reticulum and peroxisomes as other sources (Phaniendra, Babu Jestadi & Periyasamy, 2015). A variety of exogenous sources are also widely implicated in the creation or supply of ROS and include environmental effects such as smoke, heavy and transition metals, radiation, pesticides, and air pollution as well as lifestyle choices such as alcohol, tobacco use or use of certain drugs (such as paracetamol) (Phaniendra, Babu Jestadi & Periyasamy, 2015).

Nonetheless, the relationship between ROS and cancer biology is one that almost appears self-contradictory. The paradox of ROS and its effects on normal and cancerous tissues often

appears to invert depending upon conditions. The effect of DNA mutation on the part of ROS's is well characterized to increase the likelihood of cancer proliferation. However, a number of chemotherapeutic treatments employ species that augment cancer cells' existing levels of oxidative stress in attempt to abate the disease (Schumacker, 2006). Such treatments function by causing ROS levels to surpass a survivable limit and cause cell death in their excess, ideally causing cessation or reduction of the cancer (Schumacker, 2006). A preliminary excess of ROS may cause the onset of cancer, though its augmentation serves as a possible mechanism of inducing apoptosis and combatting the disease. As such, treatments to reduce ROS levels within the cell hold better roles as prevention methods, and those which increase ROS levels as a form of treatment (Sullivan & Chandel, 2014).

## Essential Oils in Cancer Care

### Current State of Essential Oils in Medicine

In some senses, EOs have already taken the stage in the world of cancer treatment, though their role continues to evolve as it has over the last several thousands of years since their use became popular. Though many will contend that some current uses of EOs in this field have ambiguous definitions or mechanisms, current implementations of EOs in this field include relieving fatigue, reducing anxiety, improving emotional stress, and alleviating pain or muscular tension (Boehm, Büssing & Ostermann, 2012). Forms of use typically include topical application, dilution and use in massage therapy, inhalation via use of an atomizer or humidifier, and even ingestion in teas or some foods (Boehm, Büssing & Ostermann, 2012).

Developments in the middle of the twentieth century brought improvements to the field of organic chemistry which made synthetic production of medicines more prevalent, causing a reduction in the medicinal use of EOs (Shaaban, El-Ghorab, Shibamoto, 2012). However,

consumer concern over synthetic compound toxicity has caused an increased interest in natural products with antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic activities (Shaaban, El-Ghorab, Shibamoto, 2012).

In light of the prevalence and cost of cancer care, and the widespread interest in EOs, it is worth investigating the possible implementation of expanding EO use beyond these listed uses. This literature seeks to summarize current knowledge and investigate some specifics surrounding chemoprevention and suppression of cancer using EOs of various plants as a complementary in oncology. In short, EO use in oncology may be considered a twofold approach: chemoprevention and cancer suppression. Much research has been conducted to clarify possible roles of EOs in both of these oncological applications with the interest of identifying ways in which EOs may prevent (chemoprevention) or suppress cancer cell growth. Such studies will be discussed in this literature.

## Cancer Suppression and Essential Oils

**Essential Oils and Induction of Apoptosis.** Programmed cell death is a normal part of life in any organism responsible for the destruction of up to 70 billion cells per day in the average adult (Reed, 1999). Failure to undergo programmed cell death when appropriate, by either apoptosis or autophagy, fosters tumorigenesis by extending life spans of damaged cells and offers greater opportunity for mutagenic activity to manifest and spread (Reed, 1999). As a result, methods of inducing apoptosis are commonly pursued as a means to combat cancer and comprise a large number of currently existing treatments. As such, these apoptotic methods are an avenue of research that has been assessed in light of EO application (Pavithra, Sreevidya & Verma, 2009).

One such study sought to illuminate apoptosis induction using EO derived from *Pamburus missionis*- a thorny shrub indigenous to India and Sri Lanka (no common name) (Pavithra, Sreevidya & Verma, 2009). This plant had been previously investigated for the antimicrobial effects conferred by its ability to cause outer membrane disintegration and lysis of the cytoplasmic membrane (Pavithra, Sreevidya & Verma, 2009; Helander et al., 1998). These characteristics of *P. missionis* then led investigators to investigate apoptotic applications.

Chemotherapeutic use of this plant's EO was assessed on solid and leukemic cancer cell lines, including cancers of the colon, breast, and skin. Solutions of cells were subjected to varied concentrations of the EO and cytotoxic activity assessed via MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Pavithra, Mehta & Verma, 2018). Nuclear morphology was assessed following treatment with EO, and progression through the cell cycle and apoptotic activity were analyzed via flow cytometry (Pavithra, Mehta & Verma, 2018).

A number of significant results were garnered from this experiment. In some cell lines, EO treatment was consistently shown to induce DNA fragmentation- a hallmark of apoptosis (Pavithra, Mehta & Verma, 2018). Additionally, flow cytometry data suggested EO treatment resulted in cell death through both early and late apoptosis (Pavithra, Mehta & Verma, 2018).Furthermore, an increase of up to 88% in ROS quantity within cells was observed 72 hours after EO treatment- a trend found to occur in multiple cell lines studied here (Pavithra, Mehta & Verma, 2018). Increase in ROS concentration within the cell is commonly observed during apoptosis, and thus ROS increases may serve as a reliable indication of the process (Jeong & Joo, 2016). As previously discussed, observation of this apoptotic occurrence has led to the pursuit of pharmaceuticals with the ability to increase intracellular ROS (such as cisplatin) to serve as a possible means of combatting tumorigenesis, even though it also serves as source of its

initial onset (Jeong & Joo, 2016). As such, the multifaceted effects of the *P. missionis* EO led researchers in this study to suggest its use in the treatment of epidermoid skin cancer cells, though such implementation would require further investigation (Pavithra, Mehta & Verma, 2018). This experiment was performed on cell cultures which provide an excellent view into the molecular biology of EO use in oncology but cannot yet fully reflect its performance within the human body, or *in vivo*.

Another EO that has been investigated in light of apoptotic mechanisms of cancer treatment is derived from gum resins of the Burseraceae tree family, namely *Boswellia sacra*. In their 2011 publication, Suhail and colleagues discovered the ability of *Boswellia sacra* EO to induce localized cytotoxicity and apoptosis specified to tumor tissues (Suhail et al., 2011). Human breast cancer cell lines were used for this research and were treated with 1:1,600 dilutions of the EO distillate. Elevated levels of cell-death shortly after treatment, assessed via quantification of lactate dehydrogenase release, were observed in all cell lines used when compared to controls (Suhail et al., 2011). Following this assay, researchers further assessed apoptosis induction following EO treatment, and found that DNA fragmentation was widely prevalent in treated cells (Suhail et al., 2011). DNA isolated from treated cells yielded a phenomenon known as DNA laddering following electrophoresis- an occurrence observed in the case of DNA fragmentation and production of a gamut of different-sized fragments (Suhail et al., 2011). Apoptotic induction of a number of nuclear endonucleases produces small DNA fragments which, when resolved in a gel, form the basis of what are known as ladder assays; these assays have become widespread as a means of fast apoptosis-screening due to their ease of use and relatively low cost (Saadat, Saeidi, Vahed, Barzegari, & Barar, 2015). This breakdown

of genomic DNA renders a cell unable to survive or proliferate, and thus serves as a reliable preliminary measure of apoptosis.

Furthermore, this research looked at levels of caspase-8 and caspase-9, members of the cysteine protease family widely implicated in apoptotic pathways (Suhail et al., 2011). Within one hour of EO treatment, elevated levels of both caspases were found in some human breast cancer cell lines used for this assessment, though the elevated levels were not detected in all cell lines (Suhail et al., 2011). Although it would be ideal to find an EO whose function is ubiquitous in all types of cancers, the need for unique approaches for the many types of cancers is illuminated by this result. This helps to highlight the treatment opportunities that may be found in EO exploitation, though also serves as a reminder that EOs cannot serve as a fix-all or universal treatment. Rather, their promising effects are best considered an extension of current methods.

Caspase-3 is also outlined in this study as the agent either mostly or entirely responsible for the cleavage of several targets involved in apoptosis; within one hour of treatment with *Boswellia sacra* EO, levels of caspase-3 were also observed to have increased in one cell line, though not in all cell lines used (Suhail et al., 2011). In addition, one of the targets of caspace-3, poly (ADP-ribose) polymerase (PARP), was found to be cleaved and inactivated over the same time frame (Suhail et al., 2011). PARP represents a family of proteins with functions ranging from catalyzing transfer of ADP-ribose onto select proteins to the chromatin structure modulation and DNA repair (Morales et al., 2014). Cleavage of PARP reduces the ability of the cell to repair DNA damage during the onset of apoptosis, further reducing the cell's chances of survival and increasing the likelihood of successful apoptosis (Morales et al., 2014). As a result,

an increase of caspase-3 supplements mechanisms of cell death and may serve as a synergistic EO-based treatment strategy.

To further elucidate accurate *in vivo* effects of EO treatment on cancer cells, self-assembled tumor spheroids were assessed following treatment. It was observed that the treatment inhibited spheroid development, a major feature of cancer malignancy, via disruption of cellular network formation (Morales et al., 2014). This test showed the translational relevance of EO treatment from cell culture solutions in 96-well plates into a form that is more clinically applicable.

However, the apoptosis-inducing effects currently found in EO-treatment are not exclusive to DNA damage. In one study by Frank et al., frankincense oil was shown to reduce viability of bladder transitional carcinoma cells in the absence of damage to the genome; rather, there was an apparent alteration of expression of a number of genes implicated in cell cycle arrest, apoptosis and growth suppression instead (Frank et al., 2009). CDKN1A (cyclin-dependent kinase inhibitor), DEDD2 (a transcription factor) and TNFAIP3 (a tumor necrosis factor) are three of the pro-apoptotic and growth-suppressive genes whose expression was increased following EO-treatment (Frank et al., 2009). Overall, 30 different genes associated with these processes were reported to have been upregulated by the EO (Frank et al., 2009). Furthermore, the study showed the effects were only observed in cancer cells without affecting the surrounding, noncancerous tissues (Frank et al., 2009).

The cancer-suppressive mechanism of action found in the case of frankincense oil is drastically different from that which was seen in a number of previously described oils. The discriminatory effect observed with frankincense EO also demonstrated the ability to selectively treat bladder cancer cells while leaving normal urothelial cells untouched. Frank et al. (2009)

conclude their results require further investigation into other bladder cancer cell lines, however, the consistency of their results with other studies may suggest a clinical application as a complementary intravesical treatment (treatment supplied via catheterization into the bladder) for this type of cancer (Frank et al., 2009). Further characterization of this oil and technique may also provide other applications in treatments of cancers found in other regions of the body.

It should be noted that this study does not suggest a full replacement of current therapies (chemotherapy and immunotherapy) with EO-treatment but proposes the treatment as a possible alternative in the future (Frank et al., 2009). However, this literature seeks to outline possible use of EO in oncology, not just as a replacement of current therapies, but also as an addition or compliment to them to improve their outcomes if possible.

**Essential Oil-Induced Cytotoxicity.** Similar to the apoptotic approach to combatting cancer, cytotoxicity is another viable avenue of treating existing cancers and may serve as another means by which EOs could be applied oncologically. While cytotoxicity may lead to the onset of apoptosis, the term itself pertains to a state of toxicity to the cell which may hinder the cell's growth, function, reproduction or overall viability (Weyermann, Lochmann & Zimmer, 2005). Whereas apoptosis assays may assess damage to cells (such as DNA fragmentation), cytotoxicity assays generally assess parameters related to viability and normal function such as metabolic activity, ATP production, or cell membrane integrity (Weyermann, Lochmann & Zimmer, 2005).

A number of EOs have been studied and observed to exhibit significant cytotoxic effects, such as EO of *Pulicaria jaubertii, Cymbopogon citratus, Porcelia macrocarpa,* and *Xylopia frutescens* (Gautam, Mantha & Mittal, 2014). Amidst the studies which observed the cytotoxic effects of these plant extracts also came elucidation of a number of oil constituents whose

cytotoxic effects in *in vivo* studies on cancer cell lines have been well-characterized. These include geraniols, perillyl alcohol, d-limonene, thymol, citrine, and a number of other compounds (Gautam, Mantha & Mittal, 2014). However, their passage into clinical applications is relatively limited; whereas perillyl alcohol has passed through some early stages of human clinical trials, many of these chemicals have not yet even begun to see clinical applications yet (Gautam, Mantha & Mittal, 2014).

One 2017 study explored cytotoxicity in extracts of *Lippia citriodora* found in various regions of Morocco. The study showed the EO to have strong cytotoxic effects on a murine mastocytoma cell line at relatively low concentrations ($IC_{50}$ ranging 7.75 to 13.25 µg/mL, compared to other statistically significant concentrations as high as 90 µg/mL discussed in this review) (Oukerrou, Tilaoui, Mouse, Bouchmaa & Zyad, 2017). The information of interest in this study was not just cancer suppression via apoptosis, but also suppression via reduction of cellular activity and viability.

Similar results were observed and published in *Toxicological Research* by Lee in 2016. α-zingiberene, ar-curcumene and β-sesquiphellandrene comprise the greater quantity of ginger essential oil in Lee's study, of which α-zingiberene was of special interest for its apparent cytotoxicity (Lee, 2016). Two cervical cancer cell lines, HeLa and SiHa, as well as breast cancer cell line MCF-7 and leukemia cell line HL60 were used for cytotoxicity screening in which 81.5% and 86% inhibition were observed, respectively, in the presence of 200 µg/mL α-zingiberene, with a spectrum of other inhibition percentages observed in a dose-dependent pattern (Lee, 2016). The MTT metabolic activity assay was used here to probe cell viability over time following treatment. The MTT assay is a type of colorimetric cytotoxicity screen in which regular metabolic activity of cells reduces a salt (3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide in this case) into a blue product, formazan, which can be measured spectrophotometrically to quantify metabolism and assess cell viability (Weyermann, Lochmann, Zimmer, 2005).

Among Lee's most interesting findings was that the $IC_{50}$ value (the amount of a drug required to halve the activity of a biological process) of α-zingiberene alone was actually significantly higher than that of entire ginger EO in all cell lines assessed, perhaps due to the presence of other chemical constituents with similar effects or synergistic activity between constituents (Aykul & Martinez-Hackert, 2016; Lee, 2016). Therefore, while it may seem desirable to identify active compounds within EOs and purify or manufacture these components alone as treatment additives, results such as these suggest that a degree of effectiveness may be lost in doing so. The complex chemical fingerprints of each EO may have more to offer than currently understood.

Cisplatin, a powerful chemotherapeutic medication, was used as a control for this viability experiment. In all cell lines studied, entire or "general" ginger EO was found to produce inhibitions more similar to cisplatin than α-zingiberene alone, except in the case of SiHa cells, in which the EO actually held an $IC_{50}$ value approximately 20 µg/mL less than that observed with cisplatin (Lee, 2016). Although this result alone certainly could not serve to support ginger EO as a replacement to cisplatin, it may be interpreted that the consistent data provided here suggests this extract may be a viable addition to current treatment methods if a functional clinical application were developed and these laboratory results able to translate to the organism-level.

Cytotoxicity is not a foreign concept in the field of EO research. Gautam, Mantha and Mittal alone report over fifty EOs for which varied levels of cytotoxicity has been a major finding in other researchers' publications (2014). The quantification also delineates cytotoxicity

separately from antiproliferative activity and inhibitory activity, listing dozens of other EOs under these categories as well (Gautam, Mantha & Mittal, 2014). As mentioned, the phenomenon of cytotoxicity is a separate entity from apoptosis, yet greatly related to it. Thus, a proper look at possible implementations of EO in cancer suppression requires assessment of effects on both. As shown by a number of researchers investigating a variety of oils from various plant sources, the cytotoxic effects mediated by EO mirror apoptotic effects previously described, signifying a possible promising clinical application.

**Essential Oils and Chemoprevention**

***Antioxidant Effect.*** Use of EO alongside current practices to combat existing cancers is an incontrovertibly important application of the natural product. Nonetheless, extension of their use into the realm of prevention is another face of this coin which warrants significant attention. Along that vein, researchers have investigated the antioxidant activity and cancer-preventative effects of a number of EOs in recent years.

One such investigation was conducted focusing on antioxidant effects, among other activities, of EO extracted from *Cnidium officinale* and *Ligusticum chuanxiong.* Jeong et al. concluded EOs from these two plants may counteract ultraviolet B-induced damage to DNA and reduce apoptosis via their constituents' significant abilities to quench free radicals (Jeong et al., 2009). Reducing the opportunity of these free radicals to react with cell components reduces the risk of events such as double-strand DNA breaks and subsequent mutations or chromosomal rearrangements which amplify carcinogenicity (Jeong et al., 2009). Of course, UVB-induced damage mainly afflicts the skin, though cancers which begin in the skin may quickly metastasize and become life-threatening. Additionally, estimations posit that the lifetime risk of developing

invasive melanoma has risen from 1 in 1,500 in the 1930's to 1 in 74 as of recently; these details are increasingly pertinent (Rigel & Carucci, 2008).

EOs distilled from *C. officinale* and *L. chuanxiong* were tested for their abilities to scavenge for free radicals and ROS. The ABTS and DPPH assays are two commonly employed methodologies used to assess antioxidant potential of a given compound or reagent (EO in the case of this experiment) in which a stable free radical chromogen species is exposed to the possible antioxidant and its irreversible reduction by the antioxidant can be detected by a color change detected via spectrophotometry (Kedare & Singh, 2011). These assays were carried out using the aforementioned plant extracts to investigate dose-dependent radical scavenging ability in various EOs, with abilities ranging from 2% activity (measured as the proportion of DPPH or ABTS radical quenched and decolorized, analyzed spectrophotometrically) at concentrations of 0.32 µg/mL to as high as 95% activity at 40 µg/mL (Jeong et al., 2009). Although this test was conducted *in vitro*, and thus cannot fully represent actual *in vivo* events, the results shed light on the chemical properties of these EOs and suggest they may possess similar activity in the greater framework of a cell, tissue, and organism. The similarities in chemical composition between oils also brings with it the possible translational nature of these results among various EOs.

Further characterization of the protective effects of these EOs was found in DNA migration assays, which allowed extension of the previous test into the context of an entire cell. The migration assay used in this study was very similar to the phenomenon of DNA laddering previously discussed. Cells were exposed to UVB light in the presence of each separate EO and in the absence of either. Gel electrophoresis of DNA isolated from these cells showed dramatic fragmentation of DNA in UVB-exposed cells in the absence of either EO compared to those cells in the presence of EO (Jeong et al., 2009). The dramatic reduction of DNA damage following oil

treatment further elucidated the antioxidant and protective nature of the extracts of *C. officinale* and *L. chuanxiong*.

The promising results of the antioxidant activity of *C. officinale* and *L. chuanxiong* in the previously described study point to a possible use of their EOs as preventative measures. As damage to DNA sets off the process of carcinogenesis, the reduction or prevention of this deleterious event confers the ability to reduce or evade the onset of cancer development. Another study brings this investigation a step further and demonstrated the ability of monoterpene perillyl alcohol to inhibit skin photocarcinogenesis in mouse models following topical application prior to UVB exposure (Pavithra et al., 2019). Monoterpene perillyl alcohol is a major component of a number of EOs, including peppermint, spearmint, cherries, sage, gingergrass, caraway, and other plants (Pavithra et al., 2019). The study showed a significant delay in tumor appearance, as well as a 25-35% reduction in overall melanoma incidence in treated mice (Pavithra et al., 2019). Though this study explored different essential oils than the previous study, it pulls the antioxidant and mutagen-combatant effects of EOs from the domain of reactions in 96-well plates into more clinically applicable context of murine studies.

However, it would be unwarranted to assume the auspicious results demonstrated here were a perfect representation of all EOs. Of course, plants vary greatly in respect to many aspects- size, shape, growth conditions, aroma, taste, and a plethora of other qualities. Thus, their EOs likely vary in a similar manner, even with certain compounds (such as various terpenes) being common from oil to oil.

An understanding of an even broader scope of antioxidant activity in a wider range of EOs may be garnered from other similar studies. The free radical scavenging ability of a variety of EOs from a variety of plant sources were analyzed in one such study, also implementing the

DPPH assay alongside two other radical-scavenging assays: the beta-carotene bleaching test and the PCL method. These three methods were selected for their high performance in assessing the activities of both lipophilic and hydrophilic compounds which allows a more accurate comparison between oils even though they may vary in their compositions (Sacchetti et al., 2005). While this study highlighted *Cymbopogon citratus* (lemon grass) EO as particularly high-performing in antioxidant activity (as well as antimicrobial activity) a number of other EOs also showed high percentages of radical-scavenging activity, including oils from *Cananga odorata, Rosmarinus officionalis* (rosemary), and *Curcuma longa* (turmeric) (Sacchetti et al., 2005). This study probes a single characteristic across a wide scope of EOs and illuminates the large number of oils with promising antioxidant activity. Although the study showed relatively low activity in some extracts, a key trend is still visible in this study; the antioxidant ability of EOs is not an isolated incidence in one oil and the potential of these extracts to prevent cellular damage is seemingly significant and thus clinically relevant (Sacchetti et al., 2005).

Translational modulation of genes implicated in antioxidant activity is another area in which EO use may have profound impact upon reduction of oxidative stress caused by ROS (Sporn & Liby, 2012). Nuclear factor erythroid 2 factor 2 (Nrf2) is a transcription factor which mediates the expression of a number of antioxidant products used to combat oxidative damage within the cell (Sporn & Liby, 2012). By binding to sequences known as antioxidant response elements on DNA, Nrf2 regulates more than 100 genes producing effects such as reduction of nitric oxide synthase expression (reducing levels of this molecule in the cell) or induction of catalase expression (an enzyme which breaks down hydrogen peroxide) (Sporn & Liby, 2012). Among other protein inductions are those of glutathione, a major electrophile scavenger and important protector of the cell from oxidative stress (Sporn & Liby, 2012). As such, the Nrf2

pathway is implicated in a variety of cellular activities, including protection from xenobiotics, normal homeostatic regulation, apoptosis and inflammation-response (Sporn & Liby, 2012).

Nrf2-knockout mice show increased incidence of cancer development and serve as a testament to this pathway's involvement in tumor suppression (Sporn & Liby, 2012). Nrf2 activation produces increased chemopreventive action by the proteins produced by the genes the pathway mediates; Nrf2 has thus been well studied in the context of cancer research and is an important facet of possible EO use in oncology as well (Sporn & Liby, 2012).

Oregano EO was shown in a 2016 study using porcine intestinal cells to upregulate a number of genes mediated by Nrf2, including superoxide dismutase 1 (SOD1), gamma-glutamylcysteine ligase (GCLC and GLCM) and catalase (CAT), each of which holds a different role in antioxidant activity within the cell (Zou, Wang, Peng & Wei, 2016). In the case of GCLC, GLCM and SOD1, mRNA expression was observed to increase in a dose-dependent manner with treatment concentrations of the EO ranging from 2.5 to 10 μg/mL (Zou, Wang, Peng & Wei, 2016). Furthermore, the Nrf2 dependency of the observed genetic modulation was demonstrated via Nrf2 downregulation using Nrf2 siRNA to reduce its mRNA expression to approximately half of its normal level; the previously described increases in SOD1, GLCM and GCLC mRNA expression were subsequently reduced following Nrf2 knockdown, highlighting their dependency on the protein's mediation in their own expression (Zou, Wang, Peng & Wei, 2016).

Similar results were observed in another 2016 study on red ginseng EO in which increased nuclear localization of Nrf2 (indicative of increased gene-regulatory activity) and upregulation of a number of genes was observed (Bak et al., 2016). Heme oxygenase 1 (HO-1), an antioxidant enzyme involved in heme catabolism, and NADPH-Quinone dehydrogenase 1 (NQO1), an enzyme which reduces and detoxifies quinones and their derivatives, are among the

genes for which increased protein expression was observed via Western blotting (Bak et al., 2016). These proteins are considered phase II antioxidant enzymes needed for carcinogen detoxification, which is believed to be mediated by the Nrf2 pathway through Nrf2 binding to the antioxidant response elements found in their genes' promoters (Bak et al., 2016).

Whereas normal physiological conditions keep Nrf2 relatively inactive by binding to a protein called Keap1, stimulation (such as oxidative stress) causes dissociation and translocation of Nrf2 to the nucleus where it binds the response elements in gene promoters and increases their expression (Bak et al., 2016). In a manner similar to that with the oregano EO, tests were conducted to link the Nrf2 pathway to these phase II enzymes. A luciferase assay using the antioxidant response element sequence as the sequence of interest was conducted and showed increased luciferase activity at higher EO concentrations (Bak et al., 2016). Additionally, increased EO concentrations yielded increased concentrations of nuclear Nrf2 protein (detected by Western blotting), and a decrease of cytoplasmic Nrf2 protein, suggesting increased translocation of the protein with higher doses of the EO (Bak et al., 2016). Nrf2 protein levels from whole cell lysate increased with dose increases as well, showing an overall protein upregulation in addition to its translocation (Bak et al., 2016). Collectively, this data suggested a strong link between the Nrf2 pathway and its regulation of these antioxidant and cell-protective enzymes as a result of red ginseng EO treatment (Bak et al., 2016).

**Anti-metastatic**. Amidst the desire to prevent and treat cancers is also found the desire to prevent its spreading via metastasis. Local effects on the primary tissue affected by a tumor certainly carry the weight of their possible effects and inhibitions of normal physiology in the area they are found, but metastasis amplifies these effects and brings with it the dangers of multi-

systemic dysfunction. In the interest of preventing this event's occurrence, it is worth elucidating EOs possible use as antimetastatic agents in a clinical context.

A study by Asif et al. (2016) demonstrated apparent anti-metastatic effects on the part of EOs extracted from *Illicum verum* (star anise) plants when used to treat a human colorectal carcinoma cell line. Namely, researchers sought to break down EO effects based on the apparent changes in three distinct stages of metastasis: local infiltration and invasion, transendothelial migration and formation of colonies and proliferation in new tissues (van Zijl, Krupitza & Mikulits, 2011).

Asif et al. (2016) first demonstrated a dose-dependent reduction in cell migration (2016). Human carcinoma cells were grown on 6-well plate and scratched with micropipette tips once confluent to create a "wound," or a gap in the cell monolayer. Different wells were subsequently treated with various concentrations of EO ranging from 25 to 90 µg/mL or 5-fluorouracil (5-FU) as a positive control. 5-FU is a fluoropyrimidine antimetabolite used in the treatment of cancers, namely colorectal cancers, which acts via inhibition of thymidylate synthase (Longley, Harkin & Johnston, 2003). Photography of the "wounds" at 0 and 24 hours allowed assessment of cell migration to fill the gap to serve as a method of investigating metastatic ability of this cell line following EO treatment.

At 24 hours, the negative control (cells treated with DMSO; no EO) had fully closed the gap, whereas cells treated with EO failed to do so (Asif et al., 2016). 25 µg/mL EO treatment yielded approximately 10% inhibition of closure, and thus a relatively low amount of anti-metastatic activity; 90 µg/mL EO treatment produced a higher level of inhibitory activity with 46% inhibition of closure (Asif et al., 2016). Treatment with 5-FU produced 81% inhibition, which is notably larger than the inhibition by EO (Asif et al., 2016). Nonetheless, 5-FU's

established use as an anticancer drug allows it to serve well as a positive control and would be expected to hinder growth and migration significantly. Although the level of inhibition observed with the high dose of EO was less than that observed with the anti-cancer drug (46% versus 81%), the dose-dependent inhibition that was nevertheless detected points to promising use of EO in pursuit of anti-metastatic treatments.

Asif et al. (2016) used a similar approach to investigate colony formation and cancer's ability to proliferate in distant tissues following metastasis. This investigation explored the role EO treatment may play in reducing this event and its effects by exposing cells to EO and then assessing their ability to grow into colonies thereafter (Asif et al., 2016).

Results of this experiment showed a high degree of similarity of EO treatment to the positive control, 5-FU, which showed almost 99% inhibition of colony formation (99% of cells were unable to grow into clusters of more than 50 cells) (Asif et al., 2016). Whereas 25 µg/mL EO-treatment produced approximately 18% inhibition of colony formation, 90 µg/mL treatment presented with 80% inhibition, closely mirroring the effects observed with 5-FU (Asif et al., 2016). While further studies would certainly be needed to improve purity of EOs and develop and refine their application techniques for future use (such as topically, injection, infusion, etc.), the results of Asif et al. (2016) show a promising possible means of reducing or arresting tumor metastasis at multiple steps of its invasive process.

## Conclusion

Cancer research is an ever-prevalent and ever-important field of investigation into which billions of dollars flow each year. The face of oncology is continuously changing, and in recent years researchers and patients alike have taken on a new interest in EOs as a possible means of natural, low-cost and scientifically based methods of treatment in the modern era. This interest

has, understandably, resulted in innumerable *in vitro* studies into antioxidant, anti-metastatic, cytotoxic and apoptotic effects mediated by EOs in attempts to illuminate ways in which these extracts may be applied medicinally in the prevention and treatment of cancer. As research moves forward, more and more *in vivo* studies are seeing the light and offer hope of upcoming clinical applications of these oils alongside current treatment methods.

EOs are complex in nature due to their unique chemical constituents, which vary even based on growth conditions. Similarly, cancer is an incredibly complex disease for which a complex treatment must be molded. Although current applications of EO in oncology are currently fairly limited and are mostly confined to aromatherapeutic and means of relief as of now, a number of studies which have helped clarify the biochemical actions EOs have within the cell point to promising chemopreventative and cancer suppressive uses for the natural extracts.

Future research will likely continue to grow the number of *in vitro* studies in order to grow the clinical relevance of such an application. Nonetheless, studies have shown dramatic abilities in certain oils and oil constituents to inhibit uncontrolled cell growth, induce apoptosis, quench ROS and free radicals, and even affect gene expression. EOs are not intended or hoped to serve as a replacement to current therapies, but rather as a compliment. However, if the effects described here can be translated clinically, they may offer the ability to improve current diagnosis outlooks in a significant and affordable way.

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