Introduction

Essential oils are complex mixtures of aromatic compounds naturally produced in plants. They have been used historically as well as currently for treating a variety of diseases and maintaining health in humans (Lv et al., 2013; Perry & Perry, 2006). Recent pre-clinical and clinical studies have provided evidence supporting the benefits of essential oils to human health (Kozioł et al., 2014; Navarra et al., 2015), resulting in a wider acceptance and use of essential oils in the US and worldwide. Despite this trend, very few studies have elucidated the mechanisms of action of essential oils in human cells.

Thousands of distinct terpene compounds have been identified in essential oils, many of which are known for possessing diverse biological activities. Because every essential oil is primarily composed of a unique mixture of just a few of these compounds, it is hypothesized that each oil has its own unique array of biological activities. For example, Oregano essential oil is known to have powerful anti-fungal and anti-microbial effects due to its high phenylpropene content, while Lavender’s main constituents, linalool and linalyl acetate, are known to calm the CNS by activating GABAA receptors.

The tendency for essential oil compounds to exhibit synergy and antagonism is another phenomenon that is receiving growing attention. A recent study on membrane dynamics suggested that the ratios of constituents might affect an oil’s activity just as much as the identity of the constituents (Hac-Wydro et al., 2017). The possibility of synergy and antagonism has sparked an interest in blending, or creating mixtures of essential oils, to achieve an oil combination with novel effects. Therefore, we studied the biological effect of an industrial essential oil blend (EOB) on a human skin disease model, the HDF3CGF pre-inflamed dermal fibroblast system, which we have used previously to study the effects of individual essential oils. The current findings will allow...
comparison of this blend’s activity with that of the individual essential oils and possibly other blends, aiding in future research on synergy, antagonism, and additive effects of essential oil blends.

The EOB contains a mixture of essential oils from frankincense (*Boswellia carterii*, *Boswellia frereana*, and *Boswellia sacra*) resin, sweet orange (*Citrus sinensis*) peel, listea (*Litsea cubeba*) fruit, thyme (*Thymus vulgaris*) plant, clove (*Eugenia caryophyllata*) bud, the summer savory (*Satureja hortensis*) plant, and niaouli (*Melaleuca quinquenervia*) leaf. Although many of these individual essential oils and their active constituents are known to have various therapeutic benefits, this was the first study to examine the effect of a commercial blend of these oils on human genome-wide gene expression in the HDF3CGF model system. We also studied the EOB’s effect on biomarkers related to inflammation, immune responses, and tissue remodeling.

**Materials and Methods**

All experiments were conducted using a biologically multiplexed activity profiling (Bio MAP) system HDF3CGF designed to model the pathology of chronic inflammation robustly and reproducibly. The system comprised three components: a cell type, stimuli to create the disease environment, and a set of biomarker (protein) readouts to examine the treatment effects on the disease environment (Berg et al., 2010). The methodologies used in this study were essentially the same as those previously described (Han & Parker, 2017a, 2017b; Kunkel et al., 2004).

**Reagents**

The EOB (dōTERRA, Pleasant Grove, UT, USA) was diluted in dimethyl sulfoxide (DMSO) to 8× the specified concentrations (final DMSO concentration was no more than 0.1%). Then, 25 μL of each 8× solution was added to the cell culture to a final volume of 200 μL while DMSO (0.1%) served as the vehicle control.

The composition of the EOB was as follows: frankincense (a mixture of *B. carterii*, *B. frereana*, and *B. sacra*) resin oil, sweet orange (*C. sinensis*) peel oil, listea (*Litsea cubeba*) fruit oil, thyme (*T. vulgaris*) plant oil, clove (*E. caryophyllata*) bud oil, summer savory (*S. hortensis*) plant oil, and niaouli (*M. quinquenervia*) leaf oil. The exact percentage composition is proprietary to the supplying company. Aromatic compounds distilled from the plant material comprised 100% of the EOB. Each essential oil originated from a country where the plant is grown. The essential oils were shipped to the US, where they were blended into the EOB. Gas chromatography-mass spectrometry analysis of the EOB showed that it contained 23–27% limonene, 11–14% alpha-pinene, 6–8% eugenol, 5–7% carvacrol, 5–7% eucalyptol, 4–6% gamma-terpinene, and smaller amounts of other aromatic compounds.

**Cell Cultures**

Primary human neonatal fibroblasts were prepared as previously described (Bergamini et al., 2012) and were plated under low-serum conditions (0.125% fetal bovine serum) for 24 h. Then, the cell culture was stimulated with a mixture of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) for another 24 h. The cell culture for the HDF3CGF assays was performed in a 96-well plate, and the stimulation conditions were described in detail elsewhere (Bergamini et al., 2012; R Development Core Team, 2011).

**Protein-Based Readouts**

An enzyme-linked immunosorbent assay (ELISA) was used to measure the biomarker levels of cell-associated and cell membrane targets. Soluble factors in the supernatants were quantified using either homogeneous time-resolved fluorescence detection, bead-based multiplex immunoassay, or capture ELISA. The adverse effects of the test agents on cell proliferation and viability (cytotoxicity) were measured using the sulforhodamine B (SRB) assay. For proliferation as says, the cells were cultured and measured after 72 h, which is optimal for the HDF3CGF system, and the detailed procedure was described in a previous study (Bergamini et al., 2012). The measurements were performed in triplicate wells, and a glossary of the biomarkers used in this study is provided in Supplementary Table S1.
# Biological Activity of an Essential Oil Blend in Human Dermal Fibroblasts

## Table S1. Glossary of Biomarkers of the Human Dermal Fibroblast System HDF3CGF Used in the Study

| Readout      | Description                                                                                                                                                                                                 |
|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CCL2/MCP-1   | MCP-1 system is a chemokine that mediates recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling. |
| CD106/VCAM-1 | VCAM-1 is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity.                                                      |
| CD54/ICAM-1  | ICAM-1 is a cell adhesion molecule that mediates leukocyte-endothelial cell adhesion and leukocyte recruitment. ICAM-1 is categorized as an inflammation-related activity.                                             |
| Collagen I   | Collagen I is involved in tissue remodeling and fibrosis, and is the most common fibrillar collagen that is found in skin, bone, tendons and other connective tissues. Collagen I is categorized as a tissue remodeling-related activity. |
| Collagen III | Collagen III is an extracellular matrix protein and fibrillar collagen found in extensible connective tissues (skin, lung and vascular system) and is involved in cell adhesion, cell migration, tissue remodeling. Collagen III is categorized as a tissue remodeling-related activity. |
| CXCL10/IP-10 | IP-10 is a chemokine that mediates T cell, monocyte and dendritic cell chemotaxis. IP-10 is categorized as an inflammation-related activity.                                                                           |
| CXCL11/I-TAC | I-TAC is a chemokine that mediates T cell and monocyte chemotaxis. I-TAC is categorized as an inflammation-related activity.                                                                                     |
| CXCL8/IL-8   | IL-8 is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity.                                                                      |
| CXCL9/MIG    | MIG is a chemokine that mediates T cell recruitment. MIG is categorized as an inflammation-related activity.                                                                                                   |
| EGFR         | EGFR is a cell surface receptor for epidermal growth factor involved in cell proliferation during development as well as tumor growth. EGFR is involved in Epithelial cell proliferation, epithelial cell differentiation keratinocyte proliferation, tissue remodeling. EGFR is categorized as a tissue remodeling-related activity. |
| M-CSF        | M-CSF is a secreted and cell surface cytokine that mediates macrophage differentiation. M-CSF is categorized as an immune modulation-related activity.                                                              |
| MMP-1        | MMP-1 is an interstitial collagenase that degrades collagens I, II and III and is involved in the process of tissue remodeling. MMP-1 is categorized as a tissue remodeling-related activity.                                |
| PAI-1        | PAI-1 is a serine proteinase inhibitor and inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) and is involved in tissue remodeling and fibrinolysis. PAI-1 is categorized as a tissue remodeling-related activity. |
| Proliferation_72hr | Proliferation_72hr in the HDF3CGF system is a measure of dermal fibroblast proliferation which is important to the process of wound healing and fibrosis.                                                          |
| SRB          | SRB is a measure of the total protein content of dermal fibroblasts. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells.  |
| TIMP-1       | TIMP-1 is a tissue inhibitor of matrix metalloprotease-7 (MMP-7) and other MMPs, and is involved in tissue remodeling, angiogenesis and fibrosis. TIMP-1 is categorized as a tissue remodeling-related activity.                                                     |
| TIMP-2       | TIMP-2 is a tissue inhibitor of matrix metalloproteases and is involved in tissue remodeling, angiogenesis and fibrosis. TIMP-2 is categorized as a tissue remodeling-related activity.                                               |
The quantitative biomarker data are presented as the mean log_{10} relative expression level (compared to their respective mean vehicle control value) ± standard deviation (SD) of triplicate measurements. Differences in biomarker levels between the EOB- and vehicle-treated cultures were tested for significance using the unpaired Student’s t-test. A p-value < 0.05, outside of the significance envelope, with an effect size of at least 10% (> 0.05 log_{10} ratio units), was considered statistically significant.

RNA Isolation

Total RNA was isolated from cell lysates using the ZymoQuick-RNA MiniPrep kit (Zymo Research Corp., Irvine, CA, USA) according to the manufacturer’s instructions. RNA concentration was determined using a Nano Drop ND-2000 system (Thermo Fisher Scientific, Waltham, MA, USA). The RNA quality was assessed using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and an Agilent RNA 6000 Nano kit. All samples had an A260/A280 ratio between 1.9 and 2.1 and an RNA integrity number score greater than 8.0.

Microarray Analysis of Genome-Wide Gene Expression

The effect of the EOB at a concentration of 0.0033% (v/v) was tested on the expression of 21,224 genes in the HDF3CGF system following a 24-h treatment. Samples for microarray analysis were processed by Asuragen, Inc. (Austin, TX, USA) according to the company’s standard operating procedures. Biotin-labeled cRNA was prepared from 200 ng total RNA using an Illumina TotalPrep RNA amplification kit (Thermo Fisher Scientific, Waltham, MA, USA) and one round of amplification. The cRNA yields were quantified using ultraviolet spectrophotometry, and the distribution of the transcript sizes was assessed using the Agilent Bioanalyzer 2100. Labeled cRNA (750 ng) was used to probe Illumina human HT-12 v4 expression bead chips (Illumina, Inc., San Diego, CA, USA). Hybridization, washing, staining with streptavidin-conjugated cyanine-3, and scanning of the Illumina arrays were carried out according to the manufacturer’s instructions. The Illumina BeadScan software was used to produce the data files for each array, and the raw data were extracted using the Illumina Bead Studio software.

The raw data were uploaded into R (R Development Core Team, 2011), and the quality-control metrics was analyzed using the beadarray package (Dunning et al., 2007). The data were normalized using quantile normalization (Bolstad et al., 2003), and then re-annotated and filtered to remove probes that were non specific or mapped to intronic or intragenic regions (Barbosa-Morais et al., 2010). The remaining probe sets comprised the data set for the subsequent analysis. The fold-change expression for each set was calculated as the log2 ratio of EOB to the vehicle control. These fold-change values were uploaded in to the Ingenuity Pathway Analysis (IPA) program (Qiagen, Redwood City, CA, USA, www.qiagen.com/ingenuity) to generate the networks and pathway analyses.

**RESULTS AND DISCUSSION**

**Bioactivity Profile of EOB in HDF3CGF System**

The HDF3CGF system was designed to model the pathology of chronic inflammation and wound healing in the context of Th1-type inflammation. Four different concentrations (0.01, 0.0033, 0.0011, and 0.0037% v/v) of the EOB were initially tested for cytotoxic activity in the dermal fibroblasts. A concentration of 0.01% was overtly cytotoxic and, therefore, the 0.0033% concentration was used in the further analysis. Biomarkers were designated if the EOB values were significantly different (p < 0.05) from vehicle controls, outside the significance envelope, with an effect size of at least 10% (> 0.05 log ratio units, Figure 1).

Studies by other research groups have shown the anti-inflammatory and immune modulating properties of the major chemical constituents of the EOB, specifically limonene and α-pinene. Topically-applied limonene, the main constituent of orange oil, reduced edema in mouse skin and pretreatment with limonene reduced inflammatory markers (Chaudhary et al., 2012). In RAW 264.7 macrophages, limonene reduced several immune markers including TNF-α (Yoon et al., 2010). Limonene's anti-inflammatory effects in rat kidney tissue were found to be associated with decreased expression of nuclear factor (NF)-κB (Rehman et al., 2014).
studies demonstrated the anti-inflammatory activities of frankincense oil and α-pinene, and the effect was likely mediated by reducing nuclear factor NF-κB nuclear translocation (Zhou et al., 2004). The immunomodulatory effect of α-pinene was attributed to the suppression of mitogen-activated protein kinases (MAPKs) and the NF-κB pathway in mouse peritoneal macrophages, which showed decreased expression of TNF-α, NF-κB, and interleukins (Kim et al., 2015).

The individual essential oils in the EOB that we have studied previously include frankincense and clove oils (Han & Parker, 2017c, 2017d). It was interesting to observe that certain biomarker effects from the individual essential oils were conserved in the blend, while others were lost. For instance, we previously found that both frankincense and clove oils significantly inhibit cell proliferation, so it was no surprise that the blend also inhibited cell proliferation. Also noteworthy was the MCP-1 downregulation by the blend, which was not observed after treatment with either of the essential oils previously studied. This difference could be attributed to one of the other oils in the blend or perhaps the unique combination of oils. Finally, the blend had virtually no effect on collagen levels, which were significantly downregulated by frankincense and clove oils. These observations support the hypothesis that the EOB has unique biological activity that may perhaps be more than a simple sum of effects from the individual essential oils included in the blend. One obvious limitation to comparing the blend with these oils, however, is our lack of biomarker data on niaouli, litsea, summer savory, thyme, and orange. Future research will make it possible to conduct a more comprehensive comparison of the effects of the EOB compared to its individual component oils.

**Fig1.** The bioactivity profile of an essential oil blend (EOB, 0.0033% v/v) in a human dermal fibroblast system HDF3CGF. Y-axis denotes the relative expression levels of biomarkers compared to vehicle control values, in log10 form. Error bars represent the standard deviations (SD) of triplicate measurements. Vehicle control values are shaded in gray, denoting the 95% significance envelope. A * indicates a biomarker designated with “key activity,” i.e., biomarker values were significantly different (p < 0.05) from vehicle controls, outside of the significance envelope, with an effect size of at least 10% (more than 0.05 log ratio units). MCP-1, monocyte chemoattractant protein; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intracellular cell adhesion molecule 1; IP-10, interferon gamma-induced protein 10; I-TAC, interferon-inducible T-cell alpha chemoattractant; IL-8, interleukin-8; MIG, monokine-induced by interferon-γ; EGFR, epidermal growth factor receptor; M-CSF, macrophage colony-stimulating factor; MMP-1, matrix metalloproteinase 1; PAI-1, plasminogen activator inhibitor 1; TIMP, tissue inhibitor of metalloproteinase.
Effect of EOB on Genome-Wide Gene Expression

To further explore the effect of 0.0033% (v/v) EOB on human skin cells, we analyzed its effect on the RNA expression of 21,224 genes. The EOB significantly regulated the expression levels of hundreds of genes globally. The vast majority of the 200 most regulated genes (178) were downregulated by the EOB while the rest were upregulated (Table S2 and see Supplementary Material for more information).

**Table S2. 200 most-impacted genes by the EOB (0.0033% v/v, fold change in log2 ratio form)**

| Illumina Gene ID | Fold Change | Definition |
|------------------|-------------|------------|
| AKR1C4           | 4.38        | Homo sapiensaldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4) (AKR1C4), mRNA. |
| MMP10            | 4.13        | Homo sapiens matrix metallopeptidase 10 (stromelysin 2) (MMP10), mRNA. |
| RSAD2            | 3.87        | Homo sapiens radical S-adenosyl methionine domain containing 2 (RSAD2), mRNA. |
| AKR1C2           | 3.66        | Homo sapiensaldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III) (AKR1C2), transcript variant 1, mRNA. XM_943424 XM_943425 XM_943427 |
| SLC7A11          | 3.17        | Homo sapiens solute carrier family 7, (cationic amino acid transporter, y+ system) member 11 (SLC7A11), mRNA. |
| ODC1             | 2.69        | Homo sapiens ornithine decarboxylase 1 (ODC1), mRNA. |
| TFRC             | 2.64        | Homo sapiens transferrin receptor (p90, CD71) (TFRC), mRNA. |
| GLDN             | 2.57        | Homo sapiens gliomedin (GLDN), mRNA. |
| TDO2             | 2.47        | Homo sapiens tryptophan 2,3-dioxygenase (TDO2), mRNA. |
| TEO2             | 2.39        | Homo sapiens TEL2, telomere maintenance 2, homolog (S. cerevisiae) (TELO2), mRNA. |
| ANGPTL4          | 2.27        | Homo sapiens angiopoietin-like 4 (ANGPTL4), transcript variant 1, mRNA. |
| MAP2             | 2.21        | Homo sapiens microtubule-associated protein 2 (MAP2), transcript variant 1, mRNA. |
| GPR1             | 2.13        | Homo sapiens G protein-coupled receptor 1 (GPR1), transcript variant 2, mRNA. |
| FAM167A          | 2.13        | Homo sapiens family with sequence similarity 167, member A (FAM167A), mRNA. |
| RPS7             | 2.13        | Homo sapiens ribosomal protein S7 (RPS7), mRNA. |
| TSPAN13          | 2.13        | Homo sapiens tetraspanin 13 (TSPAN13), mRNA. |
| SYT7             | 2.12        | Homo sapiens synaptotagmin VII (SYT7), mRNA. |
| ECE2             | 2.11        | Homo sapiens endothelin converting enzyme 2 (ECE2), transcript variant 3, mRNA. |
| C8ORF13          | 2.07        | Homo sapiens chromosome 8 open reading frame 13 (C8orf13), mRNA. |
| RPS7             | 2.06        | Homo sapiens ribosomal protein S7 (RPS7), mRNA. |
| DHRS7            | 2.05        | Homo sapiens dehydrogenase/reductase (SDR family) member 7 (DHRS7), mRNA. |
| Gene Symbol       | Fold Change | Description                                                                                                                                                                                                 |
|------------------|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ST6GALNAC3       | 2.05        | Homo sapiens ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1, 3)-N-acetylgalactosaminide alpha-2, 6-sialyltransferase 3 (ST6GALNAC3), mRNA.             |
| DIAPH3           | -2.05       | Homo sapiens diaphanous homolog 3 (Drosophila) (DIAPH3), transcript variant 1, mRNA.                                                                                                                        |
| LOC649143        | -2.05       | PREDICTED: Homo sapiens similar to HLA class II histocompatibility antigen, DRB1-9 beta chain precursor (MHC class I antigen DRB1*9) (DR-9) (DR9), transcript variant 2 (LOC649143), mRNA. |
| FOXM1            | -2.06       | Homo sapiens forkhead box M1 (FOXM1), transcript variant 2, mRNA.                                                                                                                                          |
| CCL3L3           | -2.06       | Homo sapiens chemokine (C-C motif) ligand 3-like 3 (CCL3L3), mRNA.                                                                                                                                         |
| ELF3             | -2.06       | Homo sapiens E74-like factor 3 (ets domain transcription factor, epithelial-specific ) (ELF3), mRNA.                                                                                                       |
| ADD3             | -2.06       | Homo sapiens adducin 3 (gamma) (ADD3), transcript variant 3, mRNA.                                                                                                                                       |
| HLA-DRB6         | -2.06       | Homo sapiens major histocompatibility complex, class II, DR beta 6 (pseudogene) (HLA-DRB6), non-coding RNA.                                                                                               |
| CKAP2L           | -2.07       | Homo sapiens cytoskeleton associated protein 2-like (CKAP2L), mRNA.                                                                                                                                       |
| CALD1            | -2.07       | Homo sapiens caldesmon 1 (CALD1), transcript variant 5, mRNA.                                                                                                                                             |
| FLJ21986         | -2.07       | Homo sapiens hypothetical protein FLJ21986 (FLJ21986), mRNA.                                                                                                                                              |
| ADD3             | -2.07       | Homo sapiens adducin 3 (gamma) (ADD3), transcript variant 3, mRNA.                                                                                                                                       |
| NNMT             | -2.08       | Homo sapiens nicotinamide N-methyltransferase (NNMT), mRNA.                                                                                                                                                 |
| ENPEP            | -2.08       | Homo sapiens glutamyl aminopeptidase (aminopeptidase A) (ENPEP), mRNA.                                                                                                                                    |
| NUF2             | -2.09       | Homo sapiens NUF2, NDC80 kinetochore complex component, homolog (S. cerevisiae) (NUF2), transcript variant 2, mRNA.                                                                                         |
| LYPD1            | -2.09       | Homo sapiens LY6/PLAUR domain containing 1 (LYPD1), transcript variant 1, mRNA.                                                                                                                             |
| CCNA2            | -2.09       | Homo sapiens cyclin A2 (CCNA2), mRNA.                                                                                                                                                                       |
| ANKRD22          | -2.09       | Homo sapiens ankyrin repeat domain 22 (ANKRD22), mRNA.                                                                                                                                                     |
| P4HA2            | -2.09       | Homo sapiens prolyl 4-hydroxylase, alpha polypeptide II (P4HA2), transcript variant 2, mRNA.                                                                                                               |
| COL4A2           | -2.10       | Homo sapiens collagen, type IV, alpha 2 (COL4A2), mRNA.                                                                                                                                                   |
| C10orf58         | -2.10       | Homo sapiens chromosome 10 open reading frame 58 (C10orf58), transcript variant 1, mRNA.                                                                                                                   |
| DIAPH3           | -2.11       | Homo sapiens diaphanous homolog 3 (Drosophila) (DIAPH3), transcript variant 1, mRNA.                                                                                                                      |
| CHN1             | -2.11       | Homo sapiens chimerin (chimaerin) 1 (CHN1), transcript variant 2, mRNA.                                                                                                                                   |
| FANCI            | -2.11       | Homo sapiens Fanconi anemia, complementation group I (FANCI), transcript variant 2, mRNA.                                                                                                                   |
| HJURP            | -2.11       | Homo sapiens Holliday junction recognition protein (HJURP), mRNA.                                                                                                                                           |
| ACSL5            | -2.11       | Homo sapiens acyl-CoA synthetase long-chain family member 5 (ACSL5), transcript variant 2, mRNA.                                                                                                           |
| Gene    | Expression Level | Description                                                                 |
|---------|------------------|-----------------------------------------------------------------------------|
| HLA-DRB5 | -2.12            | Homo sapiens major histocompatibility complex, class II, DR beta 5 (HLA-DRB5), mRNA. |
| BNIP3L  | -2.12            | Homo sapiens BCL2/adenovirus E1B 19kDa interacting protein 3-like (BNIP3L), mRNA. |
| LIMK2   | -2.12            | Homo sapiens LIM domain kinase 2 (LIMK2), transcript variant 1, mRNA.        |
| FBXO32  | -2.13            | Homo sapiens F-box protein 32 (FBXO32), transcript variant 2, mRNA.          |
| CCL11   | -2.14            | Homo sapiens chemokine (C-C motif) ligand 11 (CCL11), mRNA.                  |
| TMEM45A | -2.15            | Homo sapiens transmembrane protein 45A (TMEM45A), mRNA.                      |
| XIRP1   | -2.17            | Homo sapiens xin actin-binding repeat containing 1 (XIRP1), mRNA.            |
| KIF23   | -2.17            | Homo sapiens kinesin family member 23 (KIF23), transcript variant 2, mRNA.   |
| MAP1LC3A| -2.17            | Homo sapiens microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A), transcript variant 2, mRNA. |
| ENO2    | -2.18            | Homo sapiens enolase 2 (gamma, neuronal) (ENO2), mRNA.                       |
| FRMD4A  | -2.18            | Homo sapiens FERM domain containing 4A (FRMD4A), mRNA.                       |
| CXCL6   | -2.19            | Homo sapiens chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2) (CXCL6), mRNA. |
| ABCA6   | -2.19            | Homo sapiens ATP-binding cassette, sub-family A (ABC1), member 6 (ABCA6), mRNA. |
| C6ORF173| -2.19            | Homo sapiens chromosome 6 open reading frame 173 (C6orf173), mRNA.           |
| ARHGEF3 | -2.20            | Homo sapiens Rho guanine nucleotide exchange factor (GEF) 3 (ARHGEF3), mRNA.  |
| FABP3   | -2.20            | Homo sapiens fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor) (FABP3), mRNA. |
| AQP9    | -2.20            | Homo sapiens aquaporin 9 (AQP9), mRNA.                                       |
| TEX11   | -2.20            | Homo sapiens testis expressed 11 (TEX11), transcript variant 1, mRNA.        |
| CDC45L  | -2.20            | Homo sapiens CDC45 cell division cycle 45-like (S. cerevisiae) (CDC45L), mRNA. |
| IGFBP5  | -2.22            | Homo sapiens insulin-like growth factor binding protein 5 (IGFBP5), mRNA.     |
| NHS     | -2.22            | Homo sapiens Nance-Horan syndrome (congenital cataracts and dental anomalies) (NHS), transcript variant 1, mRNA. |
| ACSL5   | -2.22            | Homo sapiens acyl-CoA synthetase long-chain family member 5 (ACSL5), transcript variant 1, mRNA. |
| NDP     | -2.22            | Homo sapiens Norrie disease (pseudogioma) (NDP), mRNA.                       |
| LOC100133923 | -2.22 | PREDICTED: Homo sapiens hypothetical protein LOC100133923 (LOC100133923), mRNA. |
| KIF2C   | -2.23            | Homo sapiens kinesin family member 2C (KIF2C), mRNA.                         |
| RARRES1 | -2.23            | Homo sapiens retinoic acid receptor responder (tazarotene induced) 1 (RARRES1), transcript variant 1, mRNA. |
| MYLK    | -2.23            | Homo sapiens myosin light chain kinase (MYLK), transcript variant 8, mRNA.    |
| Gene   | Fold Change | Description                                                                 |
|--------|-------------|-----------------------------------------------------------------------------|
| SIRPA  | -2.24       | Homo sapiens signal-regulatory protein alpha (SIRPA), transcript variant 3, mRNA. |
| ROR2   | -2.24       | Homo sapiens receptor tyrosine kinase-like orphan receptor 2 (ROR2), mRNA. |
| PIM1   | -2.26       | Homo sapiens pim-1 oncogene (PIM1), mRNA.                                    |
| KIF11  | -2.26       | Homo sapiens kinesin family member 11 (KIF11), mRNA.                         |
| GRAMD3 | -2.26       | Homo sapiens GRAM domain containing 3 (GRAMD3), mRNA.                         |
| ZWINT  | -2.26       | Homo sapiens ZW10 interactor (ZWINT), transcript variant 3, mRNA.            |
| INSIG2 | -2.26       | Homo sapiens insulin induced gene 2 (INSIG2), mRNA.                          |
| NUSAP1 | -2.27       | Homo sapiens nucleolar and spindle associated protein 1 (NUSAP1), transcript variant 2, mRNA. |
| PIK3IP1| -2.27       | Homo sapiens phosphoinositide-3-kinase interacting protein 1 (PIK3IP1), mRNA. |
| CDCA5  | -2.27       | Homo sapiens cell division cycle associated 5 (CDCA5), mRNA.                |
| NCCRP1 | -2.29       | Homo sapiens non-specific cytotoxic cell receptor protein 1 homolog (zebrafish) (NCCRP1), mRNA. |
| PLAT   | -2.29       | Homo sapiens plasminogen activator, tissue (PLAT), transcript variant 1, mRNA. |
| CXCL9  | -2.29       | Homo sapiens chemokine (C-X-C motif) ligand 9 (CXCL9), mRNA.                |
| HNMT   | -2.30       | Homo sapiens histamine N-methyltransferase (HNMT), transcript variant 2, mRNA. |
| LOC100131093 | -2.30 | PREDICTED: Homo sapiens misc RNA (LOC100131093), miscRNA. |
| TACC3  | -2.30       | Homo sapiens transforming, acidic coiled-coil containing protein 3 (TACC3), mRNA. |
| DLGAP5 | -2.32       | Homo sapiens discs, large (Drosophila) homolog-associated protein 5 (DLGAP5), mRNA. |
| JAM2   | -2.32       | Homo sapiens junctional adhesion molecule 2 (JAM2), mRNA.                  |
| TPX2   | -2.33       | Homo sapiens TPX2, microtubule-associated, homolog (Xenopus laevis) (TPX2), mRNA. |
| PSTPIP2| -2.34       | Homo sapiens proline-serine-threonine phosphatase interacting protein 2 (PSTPIP2), mRNA. |
| ASF1B  | -2.36       | Homo sapiens ASF1 anti-silencing function 1 homolog B (S. cerevisiae) (ASF1B), mRNA. |
| HECW2  | -2.37       | Homo sapiens HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2 (HECW2), mRNA. |
| CLDN7  | -2.37       | Homo sapiens claudin 7 (CLDN7), mRNA.                                       |
| G0S2   | -2.38       | Homo sapiens G0/G1switch 2 (G0S2), mRNA.                                    |
| CEACAM1| -2.38       | Homo sapiens carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) (CEACAM1), transcript variant 2, mRNA. |
| HMMR   | -2.39       | Homo sapiens hyaluronan-mediated motility receptor (RHAMM) (HMMR), transcript variant 2, mRNA. |
| FAM20A | -2.39       | Homo sapiens family with sequence similarity 20, member A (FAM20A), mRNA.   |
| Gene   | Fold Change | Description                                                                 |
|--------|-------------|-----------------------------------------------------------------------------|
| CCNB2  | -2.40       | Homo sapiens cyclin B2 (CCNB2), mRNA.                                      |
| HLA-F  | -2.40       | Homo sapiens major histocompatibility complex, class I, F (HLA-F), transcript variant 1, mRNA. |
| NOD2   | -2.40       | Homo sapiens nucleotide-binding oligomerization domain containing 2 (NOD2), mRNA. |
| RARRES3| -2.44       | Homo sapiens retinoic acid receptor responder (tazarotene induced) 3 (RARRES3), mRNA. |
| STEAP4 | -2.46       | Homo sapiens STEAP family member 4 (STEAP4), mRNA.                         |
| ASCL2  | -2.47       | Homo sapiens achaete-scute complex homolog 2 (Drosophila) (ASCL2), mRNA.   |
| AK3L1  | -2.50       | Homo sapiens adenylate kinase 3-like 1 (AK3L1), nuclear gene encoding mitochondrial protein, transcript variant 7, mRNA. |
| MT3    | -2.51       | Homo sapiens metallothionein 3 (MT3), mRNA.                                |
| ACAT2  | -2.51       | Homo sapiens acetyl-Coenzyme A acetyltransferase 2 (ACAT2), mRNA.          |
| CEP55  | -2.51       | Homo sapiens centrosomal protein 55kDa (CEP55), mRNA.                      |
| ASPM   | -2.52       | Homo sapiens asp (abnormal spindle) homolog, microcephaly associated (Drosophila) (ASPM), mRNA. |
| AK3L1  | -2.52       | Homo sapiens adenylate kinase 3-like 1 (AK3L1), nuclear gene encoding mitochondrial protein, transcript variant 6, mRNA. |
| CARHSP1| -2.53       | Homo sapiens calcium regulated heat stable protein 1, 24kDa (CARHSP1), transcript variant 2, mRNA. |
| MT1F   | -2.53       | Homo sapiens metallothionein 1F (MT1F), mRNA.                              |
| CDC2   | -2.55       | Homo sapiens cell division cycle 2, G1 to S and G2 to M (CDC2), transcript variant 1, mRNA. |
| IL1A   | -2.55       | Homo sapiens interleukin 1, alpha (IL1A), mRNA.                            |
| IL4I1  | -2.57       | Homo sapiens interleukin 4 induced 1 (IL4I1), transcript variant 2, mRNA.  |
| TYMS   | -2.57       | Homo sapiens thymidylate synthetase (TYMS), mRNA.                          |
| CDKN3  | -2.58       | Homo sapiens cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase) (CDKN3), mRNA. |
| KIF4A  | -2.59       | Homo sapiens kinesin family member 4A (KIF4A), mRNA.                       |
| CDCA3  | -2.59       | Homo sapiens cell division cycle associated 3 (CDCA3), mRNA.               |
| ALDOC  | -2.59       | Homo sapiens aldolase C, fructose-bisphosphate (ALDOC), mRNA.              |
| ADAMDEC1| -2.59       | Homo sapiens ADAM-like, decysin 1 (ADAMDEC1), mRNA.                        |
| KIFC1  | -2.62       | Homo sapiens kinesin family member C1 (KIFC1), mRNA.                       |
| CD38   | -2.63       | Homo sapiens CD38 molecule (CD38), mRNA.                                   |
| NDC80  | -2.64       | Homo sapiens NDC80 homolog, kinetochore complex component (S. cerevisiae) (NDC80), mRNA. |
| Gene        | Log2FoldChange | Description                                                                 |
|-------------|----------------|----------------------------------------------------------------------------|
| MELK        | -2.64          | Homo sapiens maternal embryonic leucine zipper kinase (MELK), mRNA.         |
| HLA-DRB6    | -2.64          | Homo sapiens major histocompatibility complex, class II, DR beta 6 (pseudogene) (HLA-DRB6), non-coding RNA. |
| MMP9        | -2.65          | Homo sapiens matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) (MMP9), mRNA. |
| TP53INP2    | -2.66          | Homo sapiens tumor protein p53 inducible nuclear protein 2 (TP53INP2), mRNA. |
| BIRC5       | -2.66          | Homo sapiens baculoviral IAP repeat-containing 5 (BIRC5), transcript variant 1, mRNA. |
| SHRM        | -2.70          | Homo sapiens shroom (SHRM), mRNA.                                          |
| HLA-DRB1    | -2.70          | Homo sapiens major histocompatibility complex, class II, DR beta 1 (HLA-DRB1), mRNA. |
| RRM2        | -2.70          | Homo sapiens ribonucleotide reductase M2 polypeptide (RRM2), mRNA.          |
| SRGN        | -2.74          | Homo sapiens serglycin (SRGN), mRNA.                                        |
| MT1JP       | -2.75          | Homo sapiens metallothionein 1J (pseudogene) (MT1JP), mRNA.                 |
| AK3L1       | -2.76          | Homo sapiens adenylate kinase 3-like 1 (AK3L1), nuclear gene encoding mitochondrial protein, transcript variant 7, mRNA. |
| SLC39A8     | -2.77          | Homo sapiens solute carrier family 39 (zinc transporter), member 8 (SLC39A8), transcript variant 1, mRNA. |
| RARRES1     | -2.77          | Homo sapiens retinoic acid receptor responder (tazarotene induced) 1 (RARRES1), transcript variant 2, mRNA. |
| TK1         | -2.79          | Homo sapiens thymidine kinase 1, soluble (TK1), mRNA.                        |
| PBK         | -2.79          | Homo sapiens PDZ binding kinase (PBK), mRNA.                                |
| JUP         | -2.81          | Homo sapiens junction plakoglobin (JUP), transcript variant 1, mRNA.         |
| APOBEC3B    | -2.84          | Homo sapiens apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B (APOBEC3B), mRNA. |
| HMMR        | -2.85          | Homo sapiens hyaluronan-mediated motility receptor (RHAMM) (HMMR), transcript variant 1, mRNA. |
| DLGAP5      | -2.91          | Homo sapiens discs, large (Drosophila) homolog-associated protein 5 (DLGAP5), mRNA. |
| SCARA3      | -2.93          | Homo sapiens scavenger receptor class A, member 3 (SCARA3), transcript variant 1, mRNA. |
| C9orf135    | -2.96          | Homo sapiens chromosome 9 open reading frame 135 (C9orf135), mRNA.          |
| CYB5A       | -2.97          | Homo sapiens cytochrome b5 type A (microsomal) (CYB5A), transcript variant 2, mRNA. |
| RASD1       | -2.98          | Homo sapiens RAS, dexamethasone-induced 1 (RASD1), mRNA.                    |
| ANLN        | -2.98          | Homo sapiens anillin, actin binding protein (ANLN), mRNA.                    |
| KIF11       | -2.99          | Homo sapiens kinesin family member 11 (KIF11), mRNA.                        |
### Biological Activity of an Essential Oil Blend in Human Dermal Fibroblasts

| Gene     | Expression | Description |
|----------|------------|-------------|
| SCARA3   | -3.02      | Homo sapiens scavenger receptor class A, member 3 (SCARA3), transcript variant 2, mRNA. |
| IL32     | -3.03      | Homo sapiens interleukin 32 (IL32), transcript variant 4, mRNA. |
| HLA-DPA1 | -3.04      | Homo sapiens major histocompatibility complex, class II, DP alpha 1 (HLA-DPA1), mRNA. |
| KIAA0101 | -3.05      | Homo sapiens KIAA0101 (KIAA0101), transcript variant 1, mRNA. |
| CDC2     | -3.07      | Homo sapiens cell division cycle 2, G1 to S and G2 to M (CDC2), transcript variant 1, mRNA. |
| TNFSF10  | -3.16      | Homo sapiens tumor necrosis factor (ligand) superfamily, member 10 (TNFSF10), mRNA. |
| SRGN     | -3.21      | Homo sapiens serglycin (SRGN), mRNA. |
| SEPT4    | -3.21      | Homo sapiens septin 4 (SEPT4), transcript variant 1, mRNA. |
| MT1H     | -3.22      | Homo sapiens metallothionein 1H (MT1H), mRNA. |
| MUC1     | -3.28      | Homo sapiens mucin 1, cell surface associated (MUC1), transcript variant 6, mRNA. |
| SEPT4    | -3.29      | Homo sapiens septin 4 (SEPT4), transcript variant 3, mRNA. |
| HS.10862 | -3.32      | Homo sapiens cDNA: FLJ23313 fis, clone HEP11919 |
| SLC26A4  | -3.33      | Homo sapiens solute carrier family 26, member 4 (SLC26A4), mRNA. |
| SLC39A8  | -3.35      | Homo sapiens solute carrier family 39 (zinc transporter), member 8 (SLC39A8), transcript variant 1, mRNA. |
| IGFBP7   | -3.35      | Homo sapiens insulin-like growth factor binding protein 7 (IGFBP7), mRNA. |
| AURKB    | -3.37      | Homo sapiens aurora kinase B (AURKB), mRNA. |
| HLA-DRB4 | -3.37      | Homo sapiens major histocompatibility complex, class II, DR beta 4 (HLA-DRB4), mRNA. |
| NUSAP1   | -3.38      | Homo sapiens nucleolar and spindle associated protein 1 (NUSAP1), transcript variant 2, mRNA. |
| SAA1     | -3.39      | Homo sapiens serum amyloid A1 (SAA1), transcript variant 2, mRNA. |
| NCAPG    | -3.47      | Homo sapiens non-SMC condensin I complex, subunit G (NCAPG), mRNA. |
| UBE2C    | -3.54      | Homo sapiens ubiquitin-conjugating enzyme E2 C (UBE2C), transcript variant 3, mRNA. |
| CYB5A    | -3.55      | Homo sapiens cytochrome b5 type A (microsomal) (CYB5A), transcript variant 2, mRNA. |
| HLA-DRA  | -3.58      | Homo sapiens major histocompatibility complex, class II, DR alpha (HLA-DRA), mRNA. |
| UBE2C    | -3.60      | Homo sapiens ubiquitin-conjugating enzyme E2 C (UBE2C), transcript variant 6, mRNA. |
| HIST1H4C | -3.63      | Homo sapiens histone cluster 1, H4c (HIST1H4C), mRNA. |
| Genes/Proteins          | Log fold change | Description                                                                 |
|------------------------|----------------|-----------------------------------------------------------------------------|
| PRC1                   | -3.66          | Homo sapiens protein regulator of cytokinesis 1 (PRC1), transcript variant 2, mRNA. |
| TOP2A                  | -3.71          | Homo sapiens topoisomerase (DNA) II alpha 170kDa (TOP2A), mRNA.             |
| PALLD                  | -3.94          | Homo sapiens palladin, cytoskeletal associated protein (PALLD), transcript variant 2, mRNA. |
| HAS3                   | -3.96          | Homo sapiens hyaluronan synthase 3 (HAS3), transcript variant 1, mRNA.       |
| VCAM1                  | -4.27          | Homo sapiens vascular cell adhesion molecule 1 (VCAM1), transcript variant 1, mRNA. |
| LOC730415              | -4.42          | PREDICTED: Homo sapiens hypothetical LOC730415, transcript variant 2 (LOC730415), mRNA. |
| VCAM1                  | -4.67          | Homo sapiens vascular cell adhesion molecule 1 (VCAM1), transcript variant 1, mRNA. |
| HLA-DRA                | -4.70          | Homo sapiens major histocompatibility complex, class II, DR alpha (HLA-DRA), mRNA. |
| METTL7A                | -4.71          | Homo sapiens methyltransferase like 7A (METTL7A), mRNA.                     |
| CFB                    | -4.83          | Homo sapiens complement factor B (CFB), mRNA.                               |
| CX3CL1                 | -5.09          | Homo sapiens chemokine (C-X3-C motif) ligand 1 (CX3CL1), mRNA.              |
| SEPT4                  | -5.21          | Homo sapiens septin 4 (SEPT4), transcript variant 2, mRNA.                  |
| LIPG                   | -5.24          | Homo sapiens lipase, endothelial (LIPG), mRNA.                              |
| UBD                    | -5.80          | Homo sapiens ubiquitin D (UBD), mRNA.                                      |
| HSD11B1                | -5.88          | Homo sapiens hydroxysteroid (11-beta) dehydrogenase 1 (HSD11B1), transcript variant 2, mRNA. |
| CD74                   | -6.12          | Homo sapiens CD74 molecule, major histocompatibility complex, class II invariant chain (CD74), transcript variant 2, mRNA. |
| SLC2A5                 | -6.54          | Homo sapiens solute carrier family 2 (facilitated glucose/fructose transporter), member 5 (SLC2A5), mRNA. |
| HSD11B1                | -6.77          | Homo sapiens hydroxysteroid (11-beta) dehydrogenase 1 (HSD11B1), transcript variant 2, mRNA. |
| CCL5                   | -7.10          | Homo sapiens chemokine (C-C motif) ligand 5 (CCL5), mRNA.                   |
| MYH11                  | -7.65          | Homo sapiens myosin, heavy chain 11, smooth muscle (MYH11), transcript variant SM1A, mRNA. |
| HSD11B1                | -7.87          | Homo sapiens hydroxysteroid (11-beta) dehydrogenase 1 (HSD11B1), transcript variant 1, mRNA. |
| CD74                   | -8.00          | Homo sapiens CD74 molecule, major histocompatibility complex, class II invariant chain (CD74), transcript variant 1, mRNA. |
| CCL5                   | -10.74         | Homo sapiens chemokine (C-C motif) ligand 5 (CCL5), mRNA.                   |

Further analysis showed that the bioactivity of the EO blend significantly overlapped with many canonical pathways from the literature-validated database (Figure 2). Many of these signaling pathways are closely related to the inflammatory, immunomodulatory, and wound healing processes, as well as cancer signaling in human cells.
example, the top two matched pathways were hepatic fibrosis activation and antigen presentation. The robust inhibitory effect of the EOB on these four pathways and genes support its inflammatory and immunomodulatory properties (Tables S3-S6). The observation that the EOB significantly affected pathways related to DNA damage response and cell cycle control (e.g., mitotic roles of the polo-like kinase, cyclins, and cell cycle regulation) suggests that the EOB may have an effect on cancer biology and signaling (Figure 2).

**Table S3.** Top 20 genes regulated by EOB in the Hepatic Fibrosis/Hepatic Stellate Cell Activation canonical pathway. Fold change over vehicle is shown as a log2 ratio.

| Gene Symbol | Entrez Gene Name | Illumina Probe ID | Location | Protein Type | Entrez Gene ID for Human | Fold Change Over Vehicle |
|-------------|------------------|-------------------|----------|--------------|--------------------------|--------------------------|
| TGFB3       | transforming growth factor, beta 3 | ILMN_1687652 | Extracellular Space | growth factor | 7043 | 1.666 |
| MYL5        | myosin, light chain 5, regulatory | ILMN_2203588 | Cytoplasm | other | 4636 | 1.644 |
| TIMP2       | TIMP metallopeptidase inhibitor 2 | ILMN_1721876 | Extracellular Space | other | 7077 | -1.608 |
| A2M         | alpha-2-macroglobulin | ILMN_1745607 | Extracellular Space | transporter | 2 | -1.650 |
| COL6A3      | collagen, type VI, alpha 3 | ILMN_2307861 | Extracellular Space | other | 1293 | -1.677 |
| CD40        | CD40 molecule, TNF receptor superfamily member 5 | ILMN_1779257 | Plasma Membrane | transmembrane receptor | 958 | -1.715 |
| COL13B4     | collagen, type XVIII, alpha 1 | ILMN_1806733 | Extracellular Space | other | 80781 | -1.840 |
| EDNRA       | endothelin receptor type A | ILMN_1796629 | Plasma Membrane | transmembrane receptor | 1909 | -1.883 |
| EDNRB       | endothelin receptor type B | ILMN_1751904 | Plasma Membrane | G-protein coupled receptor | 1910 | -1.909 |
| COL1A2      | collagen, type I, alpha 1 | ILMN_1785272 | Extracellular Space | other | 1278 | -1.992 |
| IGFBP3      | insulin-like growth factor binding protein 3 | ILMN_1746085 | Extracellular Space | other | 3486 | -2.035 |
| MYH10       | myosin, heavy chain 10, non-muscle | ILMN_1815154 | Cytoplasm | other | 4628 | -2.036 |
| COL4A2      | collagen, type IV, alpha 2 | ILMN_1724994 | Extracellular Space | other | 1284 | -2.102 |
| IGFBP5      | insulin-like growth factor binding protein 5 | ILMN_2132982 | Extracellular Space | other | 3488 | -2.216 |
| CXCL9       | chemokine (C-X-C motif) ligand 9 | ILMN_1745356 | Extracellular Space | cytokine | 4283 | -2.292 |
| IL1A        | interleukin 1, alpha | ILMN_1658483 | Extracellular Space | cytokine | 3552 | -2.550 |
| MMP9        | matrix metalloproteinase 9 | ILMN_1796316 | Extracellular Space | peptidase | 4318 | -2.653 |
| VCAM1       | vascular cell adhesion molecule 1 | ILMN_2307903 | Plasma Membrane | transmembrane receptor | 7412 | -4.670 |
| MYH11       | myosin, heavy chain 11, smooth muscle | ILMN_1660086 | Cytoplasm | other | 4629 | -7.654 |
| CCL5        | chemokine (C-C motif) ligand 5 | ILMN_1773352 | Extracellular Space | cytokine | 6352 | -10.744 |
### Table S4: Top 20 genes regulated by EOB in the Antigen Presentation canonical pathway. Fold change over vehicle is shown as a log2 ratio.

| Gene Symbol | Genename | Illumina Probe ID | Location | Protein Type | Entrez Gene ID for Human | Fold Change Over Vehicle |
|-------------|----------|-------------------|----------|--------------|--------------------------|--------------------------|
| PSMB8       | proteasome (prosome, macropain) subunit, beta type, 8 | ILMN_2284794 | Cytoplasm | peptidase | 5696 | -1.278 |
| IFNG        | interferon, gamma | ILMN_2207291 | Extracellular Space | cytokine | 3458 | -1.305 |
| HLA-C       | major histocompatibility complex, class I, C | ILMN_1721113 | Plasma Membrane | other | 3107 | -1.449 |
| HLA-DQA1    | major histocompatibility complex, class II, DQ alpha 1 | ILMN_1808405 | Plasma Membrane | transmembrane receptor | 3117 | -1.485 |
| PSMB9       | proteasome (prosome, macropain) subunit, beta type, 9 | ILMN_2376108 | Cytoplasm | peptidase | 5698 | -1.491 |
| HLA-DOA     | major histocompatibility complex, class II, DO alpha | ILMN_1659075 | Plasma Membrane | transmembrane receptor | 3111 | -1.498 |
| HLA-G       | major histocompatibility complex, class I, G | ILMN_1656670 | Plasma Membrane | other | 3135 | -1.598 |
| HLA-B       | major histocompatibility complex, class I, B | ILMN_1778401 | Plasma Membrane | transmembrane receptor | 3106 | -1.612 |
| HLA-A       | major histocompatibility complex, class I, A | ILMN_2165753 | Plasma Membrane | other | 3105 | -1.681 |
| MR1         | major histocompatibility complex, class I-related | ILMN_2167416 | Plasma Membrane | transmembrane receptor | 3140 | -1.697 |
| TAPBP       | TAP binding protein (tapasin) | ILMN_1742450 | Cytoplasm | transporter | 6892 | -1.722 |
| HLA-DMA     | major histocompatibility complex, class II, DM alpha | ILMN_1695311 | Plasma Membrane | transmembrane receptor | 3108 | -2.036 |
| HLA-DMB     | major histocompatibility complex, class II, DM beta | ILMN_1761733 | Plasma Membrane | transmembrane receptor | 3109 | -2.045 |
| HLA-DRB5    | major histocompatibility complex, class II, DR beta 5 | ILMN_1697499 | Plasma Membrane | transmembrane receptor | 3127 | -2.116 |
| HLA-F       | major histocompatibility complex, class I, F | ILMN_1762861 | Plasma Membrane | transmembrane receptor | 3134 | -2.402 |
| HLA-DPA1    | major histocompatibility complex, class II, DP alpha 1 | ILMN_1772218 | Plasma Membrane | transmembrane receptor | 3113 | -3.037 |
| HLA-DRB4    | major histocompatibility complex, class II, DR beta 4 | ILMN_1752592 | Plasma Membrane | transmembrane receptor | 3126 | -3.370 |
| HLA-DRB1    | major histocompatibility complex, class II, DR beta 1 | ILMN_3228688 | Plasma Membrane | transmembrane receptor | 3123 | -4.415 |
| HLA-DRA     | major histocompatibility complex, class II, DR alpha | ILMN_2157441 | Plasma Membrane | transmembrane receptor | 3122 | -4.696 |
| CD74        | CD74 molecule, major histocompatibility complex, class II invariant chain | ILMN_1736567 | Plasma Membrane | transmembrane receptor | 972 | -7.999 |
### Table S5. Top 20 genes regulated by EOB in the Mitotic Roles of Polo-Like Kinase canonical pathway. Fold change over vehicle is shown as a log2 ratio.

| Gene Symbol | Entrez Gene Name | Illumina Probe ID | Location | Protein Type | Entrez Gene ID for Human | Fold Change Over Vehicle |
|-------------|------------------|-------------------|----------|--------------|--------------------------|--------------------------|
| FZR1        | fizzy/cell division cycle 20 related 1 (Drosophila) | ILMN_3306993 | Nucleus | kinase       | 51343 | 1.389 |
| PPP2R2A     | protein phosphatase 2, regulatory subunit B, alpha | ILMN_1788961 | Cytoplasm | phosphatase | 5520 | 1.330 |
| RAD21       | RAD21 homolog (S. pombe) | ILMN_1748578 | Nucleus | other | 5885 | -1.352 |
| CCNB3       | cyclin B3 | ILMN_1705757 | Nucleus | other | 85417 | -1.361 |
| PPP2R4      | protein phosphatase 2A, activator, regulatory subunit 4 | ILMN_1658951 | Cytoplasm | phosphatase | 5524 | -1.371 |
| PTTG1       | pituitary tumor-transforming 1 | ILMN_2042771 | Nucleus | transcription regulator | 9232 | -1.407 |
| PPP2R3B     | protein phosphatase 2, regulatory subunit B’, beta | ILMN_1712257 | Nucleus | phosphatase | 28227 | -1.411 |
| PKMYT1      | protein kinase, membrane associated tyrosine/threonine 1 | ILMN_2401436 | Cytoplasm | kinase | 9088 | -1.414 |
| WEE1        | WEE1 G2 checkpoint kinase | ILMN_1778561 | Nucleus | kinase | 7465 | -1.470 |
| PLK1        | polo-like kinase 1 | ILMN_1736176 | Nucleus | kinase | 5347 | -1.531 |
| CCNB1       | cyclin B1 | ILMN_1712803 | Cytoplasm | kinase | 891 | -1.621 |
| CDC20       | cell division cycle 20 | ILMN_1663390 | Nucleus | other | 991 | -1.778 |
| FBXOS5      | F-box protein 5 | ILMN_1710676 | Nucleus | enzyme | 26271 | -1.785 |
| PLK4        | polo-like kinase 4 | ILMN_1789123 | Cytoplasm | kinase | 10733 | -1.849 |
| CDC25C      | cell division cycle 25C | ILMN_1725260 | Nucleus | phosphatase | 995 | -1.905 |
| KIF23       | kinesin family member 23 | ILMN_1811472 | Cytoplasm | other | 9493 | -2.166 |
| CCNB2       | cyclin B2 | ILMN_1801939 | Cytoplasm | other | 9133 | -2.398 |
| KIF11       | kinesin family member 11 | ILMN_2143155 | Nucleus | other | 3832 | -2.987 |
| CDK1        | cyclin-dependent kinase 1 | ILMN_1747911 | Nucleus | kinase | 983 | -3.065 |
| PRC1        | protein regulator of cytokinesis 1 | ILMN_1728934 | Nucleus | other | 9055 | -3.664 |
### Table S6. Top 20 genes regulated by EOB in the Cyclins and Cell Cycle Regulation canonical pathway. Fold change over vehicle is shown as a log2 ratio.

| Gene Symbol | Entrez Gene Name | Illumina Probe ID | Location       | Protein Type          | Entrez Gene ID for Human | Fold Change Over Vehicle |
|-------------|------------------|-------------------|----------------|-----------------------|--------------------------|--------------------------|
| E2F5        | E2F transcription factor 5, p130-binding | ILMN_1782551 | Nucleus | transcription regulator | 1875 | 1.901 |
| TGFβ3       | transforming growth factor, beta 3 | ILMN_1687652 | Extracellular Space | growth factor | 7043 | 1.666 |
| PA2G4       | proliferation-associated 2G4, 38kDa | ILMN_1728984 | Nucleus | transcription regulator | 5036 | 1.395 |
| CDKN2C      | cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4) | ILMN_2359332 | Nucleus | transcription regulator | 1031 | 1.387 |
| HDAC2       | histone deacetylase 2 | ILMN_1767747 | Nucleus | transcription regulator | 3066 | 1.337 |
| PPP2R2A     | protein phosphatase 2, regulatory subunit B, alpha | ILMN_1788961 | Cytoplasm | phosphatase | 5520 | 1.330 |
| CCNB3       | cyclin B3 | ILMN_1705757 | Nucleus | other | 85417 | -1.361 |
| CDK2        | cyclin-dependent kinase 2 | ILMN_1665559 | Nucleus | kinase | 1017 | -1.368 |
| PPP2R4      | protein phosphatase 2A activator, regulatory subunit 4 | ILMN_1658951 | Cytoplasm | phosphatase | 5524 | -1.371 |
| PPP2R3B     | protein phosphatase 2, regulatory subunit B", beta | ILMN_1712257 | Nucleus | phosphatase | 28227 | -1.411 |
| RB1         | retinoblastoma 1 | ILMN_1696591 | Nucleus | transcription regulator | 5925 | -1.411 |
| E2F2        | E2F transcription factor 2 | ILMN_1777233 | Nucleus | transcription regulator | 1870 | -1.444 |
| WEE1        | WEE1 G2 checkpoint kinase | ILMN_1778561 | Nucleus | kinase | 7465 | -1.470 |
| CDKN1B      | cyclin-dependent kinase inhibitor 1B (p27, Kip1) | ILMN_2196347 | Nucleus | kinase | 1027 | -1.479 |
| CCNB1       | cyclin B1 | ILMN_1712803 | Cytoplasm | kinase | 891 | -1.621 |
| CDKN2B      | cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4) | ILMN_2376723 | Nucleus | transcription regulator | 1030 | -1.766 |
| CCNE2       | cyclin E2 | ILMN_2412384 | Nucleus | other | 9134 | -1.827 |
| CCNA2       | cyclin A2 | ILMN_1786125 | Nucleus | other | 890 | -2.088 |
| CCNB2       | cyclin B2 | ILMN_1801939 | Cytoplasm | other | 9133 | -2.398 |
| CDK1        | cyclin-dependent kinase 1 | ILMN_1747911 | Nucleus | kinase | 983 | -3.065 |
Fig2. Top 20 canonical pathways matching the bioactivity profile of the essential oil blend (EOB, 0.0033% v/v) in gene expression in an HDF3CGF system, produced using Ingenuity Pathway Analysis (IPA). Each p-value was calculated using the right-tailed Fisher’s exact test. The p-value measures the likelihood that the observed association between a specific pathway and the dataset is due to random chance. Pathways with smaller p-values (bigger \(-\ln p\)-values), indicated by the black bars, matched the bioactivity of the EOB more significantly than those with higher values did. A ratio, indicated by each gray bar, was calculated by taking the number of genes from the EOB dataset that participate in a canonical pathway and dividing it by the total number of genes in that pathway. BRCA1, human breast cancer gene 1; THOP1, thimetoligopeptidase; AD, Alzheimer’s disease; OX40 (TNFRSF4), tumor necrosis factor receptor superfamily, member 4; p53, tumor suppressor protein 53.
These results are also consistent with findings of other research studies on the biological activity of the primary chemical constituents of the EOB. For example, limonene inhibited the expression of a number of inflammatory genes in an osteoarthritis model (Rufino et al., 2015) and α-pinene affected immunomodulatory genes in a mouse model of allergic rhinitis (Nam et al., 2014). Eugenol, the third most abundant constituent in the EOB, is known for its anti-cancer and anti-inflammatory properties in various cell types. It has been shown to upregulate genes in the base excision repair pathway (Ghosh et al., 2005) while downregulating inflammatory cytokines (Pal et al., 2010) and antiapoptotic genes (Kaur et al., 2010).

This study provides important data on the biological activity of an EOB in cytokine-stimulated human dermal fibroblasts. The data suggest that the EOB may modulate inflammatory and immune responses, tissue remodeling, and cancer signaling processes in a manner unique to any individual oil studied previously. Further research is needed to elucidate the biological and physiological mechanisms of action of the EOB and any synergistic or antagonistic interactions between the compounds contained therein.

CONCLUSIONS

To the best of our knowledge, this is the first study to analyze the effect of an EOB on genome-wide gene expression in human skin cells. The EOB significantly modulated mRNA levels of genes involved in a variety of important signaling pathways including inflammation, immune function, wound healing, cell cycle regulation, and DNA damage repair. It’s biological activity is unique to any pure essential oil studied previously. This study provides original and important data on the modulation of genome-wide gene expression in validated human cell cultures by an essential oil blend. Finally, these results suggest that this EOB may possess the potential to modulate inflammatory and immune responses in skin cells, giving it practical applications in the fields of dermatology and medicine in general.

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Competing Interest

Xuesheng Han and Tyler Bahr are employees of dōTERRA, where the studied agent EOB was manufactured.

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