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Gene expression profile analysis and identification of the effects triggered by essential sandalwood oil on human skin explants

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Sandalwood oil is an essential oil obtained from the tree wood of various species of *Santalum spp* by steam distillation, and has a history of medicinal use besides as a fragrance. However the mechanisms involved in its activity are still unclear. In the present report, Sandalwood oil (*S. album*) was applied to living human skin explants to evaluate the sustained activity of the oil through gene expression profiling. Transcriptomic analysis showed a number of metabolic pathways and biological activities triggered by treatments previously described for aloe, curcumin and luteolin. No inflammatory responses accompanied the beneficial activities. The most intriguing impact was the natural effect on the retinoic acid (vitamin A) metabolism.

Furthermore, we found that mechanisms normally acting in more specialized cell types such as immune cells, adipocytes, nerve cells, or hair follicles were activated in human skin explants in response to sandalwood oil. Thus, the oil may have the potential of further beneficial activities such as its antibacterial activity among other protective mechanisms.

These results corroborate at a molecular level the rationale for clinical studies with sandalwood oil and support recent studies of patients with eczema (atopic dermatitis) or acne, which is often accompanied by bacterial growth on skin of *Propionibacterium acnes*.

(211 words)

Key words: sandalwood oil, skin, gene activation, microarray analysis, expression profiling, transcriptome, plant extracts,

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INTRODUCTION

Sandalwood oil (SO) is an essential oil obtained from the wood of various species of *Santalum spp.* Since ancient times this essential oil served for religious purposes as an incense and used in fragrances, flavorings, and as a traditional medicine for use as an antidepressant, anti-inflammatory, anti-fungal, astringent, sedative, as an insecticide, and antiseptic (Burdock GA and Carabin IG 2008). SO is a mixture of compounds with similar structural and physical characteristics but contains primarily a sesquiterpene alcohol molecule called santalol, which has two isomeric forms, α -santalol (41-55%) and β -santalol (16-24%), both of which are volatile compounds with a unique aroma and are the primary active components. α -santalol has proved to be a potential chemopreventive agent against UVB-induced skin tumor development in mice (Dwivedi et al. 2006), while β -santalol was investigated for its antiviral activity against influenza A/HK (H3N2) virus in MDCK cells (Paulpandi et al. 2012). Additionally, purified α -santalol and β -santalol were able to suppress secretion of key cytokine-related factors and proinflammatory arachidonic acid metabolite production in skin cells. Lipopolysaccharides stimulated the release of 26 cytokines and chemokines, 20 of which were substantially suppressed by simultaneous exposure to either of the two sandalwood compounds and to ibuprofen (Sharma M et al. 2014). In a study on a cohort of radiotherapy patients, turmeric- and sandal oil-based cream was effective in preventing radiation-induced dermatitis. The damage is mainly mediated via indirect effects, where the generation of free radicals, resulting from the radiolysis of water, causes damage to macromolecules such as DNA, proteins and lipids (Palatty PL et al. 2014).

SO showed insecticidal activity with LD₅₀ values ($\mu\text{g}/\text{fly}$) of 2.18 and of 5.61 against male and female drosophila flies, respectively (Kim J et al. 2016). *In vivo* analysis using a rodent model confirmed the anti-plasmodial potential of subcutaneously administered sandalwood oil (Fujisaki R et al. 2012). It also is an effective repellent against spider mites (Roh HS et al. 2012).

Other minor molecules are derivatives such as the aldehydes santalal and cyclo-santalal, and traces of santalene, curcumene and bergamotene. The latter two terpenes can also be found in essential oils from different plants. For instance, curcuminoids from the curcuma root or sage possess anti-oxidant, anti-inflammatory and anti-mutagenic properties and protect the body from mutagens such as smoke and other pollutants (Jayaprakasha GK et al. 2005). Bergamotene itself is found in a variety of plants, from basil (Hussaina AI et al. 2007), and the Brazilian tree Pau Santo (*Kielmeyera coriacea*) to orchids (Sadler JJ et al. 2011), and has been used for its antimicrobial activity (Martins et al. 2015). Presently, seven clinical trials are under way which utilize SO in treatments of common warts, the infection molluscum contagiosum in children, caused by a poxvirus, atopic dermatitis (eczema) and acne, and oral mucositis or plaque psoriasis (clinicaltrials.org). In preliminary results treating eczema

with SO, 68% of patients achieved an IGA score of "much improved" or "very much improved" with a minimum 2-grade improvement after eight weeks of treatment (NCT02871479).

The purpose of the present study was to establish at the molecular level, a genetic profile of the biological activity of essential sandalwood oil from *Santalum album*, produced under standardized conditions. The model system under investigation is based on living human skin explants. This protocol permits to compare *ex vivo* the effects of SO at the histological level as well as the changes of gene expression as a function of SO concentration and duration of treatment. Using proprietary dedicated software, PredictSeach®, functional correlations between genes were identified allowing the generation of biological networks related to SO activities.

MATERIALS AND METHODS

The wood was obtained from managed plantations of *Santalum album* grown in the Kununurra district of Western Australia. The plantations were owned by Santanol. The essential oil was extracted from the rich heartwood of plantation trees that were 15 years old. Oil was obtained through the steam distillation of wood chips loaded into commercial size stainless steel distillation pots. The effluent was cooled and condensed into a collection vessel and the top layer containing the oil was collected. The quality of the oil complies with that set out in ISO 3518/2002 – Oils of Sandalwood (*Santalum album* L.). A typical chromatogram is shown in **Figure 1**, and the extracted SO presented a composition as shown in **Table 1**.

The study was approved by an ethics committee and performed in accordance with the Declaration of Helsinki after the patient had given informed consent. A full-thickness human skin biopsy was obtained after plastic surgery from a healthy female donor (49-year-old Caucasian). The abdomoplasty was immediately transported at room temperature in minimum essential medium 1X (MEM, Gibco life technologies™; Paisley, UK) to BIO-EC laboratory. The hypodermis was removed from the skin, and circular explants (~1cm diameter, 0.2cm thickness, 180mg) were then excised using a sample punch. Explants, dermis face down, were directly placed in a liquid–air interface in BIO-EC's Explant Medium (BEM), containing gentamicin and fungizone for 24h before treatment. Skin samples were maintained under typical cell culture conditions (37°C in 5% CO₂), and half of the medium (1ml) was refreshed every other day.

Samples were taken for each timepoint. Triplicates were removed at 9h and 24h for genomic expression analysis and histology, and on day 8 for morphological evaluation. Treatment consisted of excipient alone (sweet almond oil) as control, or 0.1, 0.2, 0.4% (P3), 1.0 and 2% (P1) *Santalum album* oil in excipient, on day 0 (D0), day 1, day 4 and day 6 on the basis of 2mg per explant.

Samples were cut in half for storage in RNAlater (Qiagen) or fixation in buffered formol. For histology, samples were dehydrated and impregnated in paraffin, and embedded according to the SOP H-153 using a Leica EG 1160 embedding station. 5-µm-thick sections were made according to the SOP H-173 with a Leica RM 2125 Minot-type microtome, and the sections were then mounted on Superfrost® Plus silanized glass slides. Microscopical observations were realized using a Leica DMLB or Olympus BX43 microscope. Pictures were digitized with a numeric DP72 Olympus camera with CellD storing software. General morphology was assessed on controls D-1, D0, and D8, and treated samples on D8 after staining of paraffinized sections according to Masson's trichrome, Goldner variant.

Total RNA was extracted with ReliaPrep Tissue Miniprep system (fibrous) from Promega. RNA (40ng) of each sample was used for reverse transcription, amplification and Cy3 labeling, using the Low Input Labeling kit, one-color (Agilent Technologies). All cRNAs were hybridized to human whole

genome microarrays (Agilent Technologies). Microarray data were quantified and normalized according to Agilent protocol (GeneExtraction Feature V10.5.5.1), and deposited in the public domain (GEO Submission GSE106734). Subsequently, the fold-change (FC) between treated versus untreated condition was calculated by the following ratios: transcript intensity of treated samples versus transcript intensity of control samples. The selection of genes of interest were identified in sequential steps: a) Controls were considered unchanged if their expression was between 0.8 and 1.25 fold-change at two time points, 9h (early) and 24h (late). b) In order to take into account the effect of the tested product (P), only gene transcripts that were not subjected to modulation caused by the excipient (almond oil) were considered. Therefore, genes with an $FC \geq 1.25$ between excipient and control values (intensities higher than background) in at least two out of three similar conditions (for each permutation between treated samples and controls), were removed.

The remaining genes were then filtered for either $FC \geq 1.25$ and < 1.45 , and for $FC \geq 1.45$ between P and control values (intensities higher than background) in at least two out of three similar conditions (for each permutation between all treated samples and controls) for either P1 (2%) or P3 (0.4%) at either one (9h or 24h) or both time points. P1 and P3 were chosen based on the histology results.

Selected genes were subjected to functional analysis by GENEX' proprietary PredictSearch® program in order to identify the induced biological effects. PredictSearch® is a powerful text mining software application that identifies correlations between genes and biological processes/diseases within all scientific publications cited in the PubMed database.

RESULTS and DISCUSSION

The selection resulted in 65 genes, which were activated as a consequence of sandalwood oil treatment. The complete absence of any inflammatory genes and few immune response-related genes indicated the oil was very well tolerated. It is important to note that for specific plant species, particular characteristics exist in their content of secondary metabolites (phenolics, alkaloids, steroids, terpenoids, acetophenones) according to the geographical location between families. Of the different *Santalum* species, the SO under investigation comes from *Santalum album*, which contains no farnesol, compared to the native Australian *Santalum spicatum* that may contain 5-30% of this contact allergen (Moniodis et al. 2017). **Figure 2** compares the morphology of explants on D8 between control or treated either with excipient or 2% essential oil. There are no discernible alterations but an increase of the epidermal layer following treatment.

The global overview shows that a retinoic acid (RA) and survival pathway, differentiation and proliferation are the predominant motives as 40 genes related to these processes, partially overlapping, are induced. Interestingly, several activities known from other essential oils or plant extracts can be found in this study, including retinoic acid-related pathways with activities similar to medicinal drugs under development. **Table 2** lists the most important genes and the induced fold-changes in this study. The overall activities are summarized in **Figure 3** and described below.

The following distinct pathways could be attributed to the induced genes: a RA signaling pathway, mechanisms concerning cell cycle control, proliferation and differentiation, including DNA repair, remodeling activities with cell structure changes, and protective activities.

Several signature genes of the RA pathway are induced after 24h with 2% SO: CRABP2 (cellular retinoic acid binding protein 2), PLIN2 (perilipin 2), and PPARG (peroxisome proliferator activated receptor gamma). The protein Crabp2 is a cytosol-to-nuclear shuttling protein. It is involved in the retinoid signaling pathway, and the classical function of Crabp2 is to directly deliver RA to its nuclear receptor (RAR), which in turn induces the expression of multiple antiproliferative genes (Vreeland AC et al. 2014). Consequently, Crabp2 might potentially act as a negative regulator that limits cellular senescence in fibroblasts (Kim YW et al. 2010, Si SP 1995). PPARs are a family of nuclear receptors, which can heterodimerize with retinoid X receptors to regulate transcription of various genes, including PLIN2 in adipocyte differentiation and lipid metabolism. The protein is the major cAMP-dependent protein kinase substrate in adipocytes and, when unphosphorylated, may play a role in the inhibition of lipolysis. (Semple RK et al. 2006). It is also a target gene of PPARG which binds directly to the PPRE site on the promoter (Kang et al. 2015).

When topically applied, RA induces keratinocyte proliferation and upregulates CRABP2, a signal transduced by RXR alpha/RAR gamma heterodimers in suprabasal keratinocytes, which, in turn,

stimulate proliferation of basal keratinocytes (Chapellier B et al. 2002, Elder JT et al. 1992). The analysis of stimulated gene expression suggests that SO treatment has a significant effect on cell growth and the differentiation processes. The signaling events that correspond to the retinoic acid pathway (CRABP2, PPARG, perilipin 2) decrease cell growth and promote differentiation. Furthermore, the drug Troglitazone®, one of the thiazolidinedione (TZD) class of anti-diabetic drugs and a ligand for PPARG, inhibits CCND1 (cyclin D1) expression. Troglitazone® and other PPARG ligands have been shown to inhibit cell proliferation and induce cell cycle arrest in a variety of cancer cells as well as to inhibit mitogen-induced cellular proliferation in normal mouse skin primary keratinocytes (He G et al. 2004).

Most interesting are activities similar to that with the drug Rambazole (Talarozole, Barrier Therapeutics, Geel, Belgium) currently under development for its interference in retinoic acid (vitamin A)-related pathways. Rambazole increases intracellular levels of endogenous all-trans RA through inhibition of cytochrome P450-dependent all-trans-retinoic acid catabolism. It is in clinical development for the treatment of psoriasis and acne (Baert B and De Spiegeleer B 2011). Well-known effects of RA are normalization of aberrant epithelial growth and differentiation. Hence, Rambazole may be utilized in the treatment of plaque psoriasis (Bovenschen HJ et al. 2007). Other skin diseases such as acne or keratinization disorders might benefit from this activity.

More genes, which code for proteins regulating proliferation and differentiation are detectable: ODC1 (ornithine decarboxylase 1), RAB1B (member RAS oncogene family) and BCL6B (B-cell CLL/lymphoma 6B), all have been shown to have tumor suppressor activity in various cancer models (Wang X and Jiang L 2014, Jiang HL et al. 2015; Wang J et al. 2014). NAMPT (nicotinamide phosphoribosyltransferase, visfatin) belongs to the nicotinic acid phospho-ribosyltransferase (NAPRTase) family and is thought to be involved in many important biological processes, including metabolism, stress response and anti-aging by activating sirtuin 1 (Tsai PJ et al. 2015). NAMPT is a rate-limiting enzyme in the NAD(+) salvage pathway of NAD(+), which serves as a transfer molecule for electrons, thereby acting as a key cofactor for energy production, an activity shared with PPP1R3 (protein phosphatase 1 regulatory subunit 3F) expressing a glycogen-binding protein (Kelsall IR et al. 2011). NAMPT activates G1-S phase cell cycle progression by upregulation of CCND1 (Kim JG et al. 2010). DDX5 (DEAD-box helicase) is essential for normal cell proliferation, and the transition from G1 to S/G2 phase is accompanied by an increase of DDX5 protein concentration in the cells (Ponomartsev NV and Enukashvily NE 2015). Two more members of the same family, DDX3Y and DDX6, were induced. The former is found in P-bodies and stress granules, and functions in translation suppression and mRNA degradation. It is required for microRNA-induced gene silencing. The latter, DDX6 is necessary for maintaining adult progenitor cell function and its loss results in premature differentiation and decreased proliferation of epidermal progenitor cells (Wang Y et al. 2015).

FGFR4 (fibroblast growth factor receptor 4) encodes a member of the fibroblast growth factor receptor family, where the amino acid sequence is highly conserved between members and throughout evolution. Functional assays have demonstrated that FGFR4 can induce proliferation, invasion, and epithelial-mesenchymal transition (EMT) after FGF stimulation. Indeed, FGFR4 inhibitors can suppress proliferation, invasion and induce apoptosis (Xu YF et al. 2014). LOXL2 (lysyl oxidase like 2) belongs to a gene family, where the prototypic member is essential for the biogenesis of connective tissue. The effect of LOXL2 on cell cycle and apoptosis-related components has been demonstrated through the silencing of LOXL2 expression, which activates the FAK/Akt/mTOR signaling pathway (Kim BR et al. 2014).

BOP1 (block of proliferation 1) has an important role in hepatocellular carcinoma invasiveness and metastasis potentials through inducing epithelial to mesenchymal transition, and promoting actin cytoskeleton remodeling. BOP1 is a novel nucleolar protein involved in rRNA processing and ribosome assembly. Kinase activities of the G(1)-specific CDK2 and CDK4 complexes were downregulated in cells expressing BOP1DELTA, whereas levels of the CDK inhibitors p21 (CDKN1A cyclin-dependent kinase inhibitor 1A) and p27 (CDKN1B cyclin-dependent kinase inhibitor 1B) were concomitantly increased (Pestov DG et al. 2001). The conserved protein Bop1 is essential for viability. Lower transcript levels result in defects in rRNA processing and developmental abnormalities that are consistent with its predicted role in ribosome biogenesis (Carvalho SD et al. 2016).

The proliferating cell nuclear antigen (PCNA) is found in the nucleus and is a cofactor of DNA polymerase delta. It acts as a homotrimer and helps increase the processivity of leading strand synthesis during DNA replication, however in response to DNA damage, this protein is ubiquitinated and is involved in the RAD6-dependent DNA repair pathway (Yin L et al. 2015). NES (nestin) is a key regulator of various extracellular proteins that play important roles in cell growth and differentiation. Overexpression promotes the embryonic development of heart and brain through the regulation of cell proliferation (Liu J et al. 2015). Nestin is found only in intramesenchymal skin, and stimulation of organ-cultured human scalp skin with the adipokine leptin increased the number of nestin⁺ cells in these intramesenchymal skin locations (Tiede S et al. 2009). The gene SRC (proto-oncogene, non-receptor tyrosine kinase Src) may play a role in the regulation of embryonic development and cell growth and its activity can be inhibited by phosphorylation through c-SRC kinase.

According to the kinetics in this study, it is possible that these effects are initiated sequentially. For instance, within the skin granular layer, proliferation of undifferentiated cells is followed by DNA repair activity (to preserve the integrity of growing cells), and growth arrest (inducing some apoptosis-related genes), which in turn allows cells to differentiate. These activities concern cellular structures such as those belonging to the cytoskeleton, and might impact the extra cellular matrix.

This notion is supported by activated genes that are involved in cellular architecture and remodeling with the help of proteins like keratins and proteases.

Cellular structural changes and remodeling activities are reflected in the increase of genes like KRT8. The protein keratin 8 plays a role in maintaining cellular structural integrity and also functions in signal transduction and cellular differentiation. ELMO1 (engulfment and cell motility 1) encodes a member of the engulfment and cell motility protein family. These proteins interact with DOCK8 (dedicator of cytokinesis) protein to promote phagocytosis and cell migration. ELMO1 is also a target of phosphorylation by Src (Makino Y et al. 2015). ADAMTS4 (a disintegrin and metalloproteinase with thrombospondin type 1 motif 4) is responsible for the degradation of specific proteoglycans (Kelwick R et al. 2015), while calpain 2 (CAPN2) belongs to calcium-activated, nonlysosomal, intracellular cysteine proteases. Calpain activity is implicated in important cellular processes including cytoskeletal remodeling, apoptosis and survival (Storr SJ et al.). Connexins such as GJP2 (gap junction protein 2) are involved in cell motility and wound healing and GJP2 enhances cell motility [Polusani SR et al. 2016].

Another facet are protective measures triggered at the molecular level, which target a host of conditions from inflammation to auto-immune disorders, infections and other defense systems.

Induced protective activities can be found in several modulated genes: DOK3 (docking protein 3), a negative regulator of toll-like receptor signaling by limiting LPS-induced ERK activation and cytokine responses in macrophages (Peng Q et al. 2012). IDO1 (indoleamine 2,3-dioxygenase 1) activity is the first and rate-limiting step in tryptophan catabolism in a variety of pathophysiological processes such as antimicrobial and antitumor defense, neuropathology, immunoregulation, and antioxidant activity (Yeung AW et al. 2015). The alpha M beta 2 integrin ITGAM (CD11b) is important in the adherence of neutrophils and monocytes to stimulated endothelium, and also in the phagocytosis of complement-coated particles. It has been shown that polymorphonuclear leukocytes play a pivotal role in early-phase immune response to bacterial or periprosthetic infection (Kim GD et al. 2014).

It is interesting to note that the antimicrobial activity may be beneficial for the treatment of acne vulgaris. Several essential oils have been used to treat acne. Proposed mechanisms for tea tree oil's anti-acne effects include antibacterial action against e.g. *Propionibacterium acnes* and anti-inflammatory properties (Hammer KA 2014). Activities of ten essential oils towards *P. acnes* have been tested directly on the bacteria but not necessarily at the molecular level (Zu Y et al. 2010).

The cell surface molecule ABCC10 (ATP binding cassette subfamily C member 10) is a broad-acting transporter of xenobiotics, localized at the basolateral cell surface, as was shown with [³H]-docetaxel (Malofeeva EV et al. 2012). Finally, sulfotransferase enzymes, such as encoded by SULT1A2, catalyze

the sulfate conjugation of many hormones, neurotransmitters, drugs, and xenobiotic compounds by increasing their solubility and facilitating their excretion (Fernández-Santander A et al. 2013).

The intriguing aspect of SO is that it shares the activities described in other medicinal plants, the active ingredients of curcumin, luteolin and aloe.

The genes SRC, CCND1, ODC1 and PCNA are genes that are equally activated by curcuminoids. Vogel and Pelletier (1815) reported the isolation of a “yellow coloring-matter” from the rhizomes of *Curcuma longa* (turmeric) and named it curcumin. In turmeric, which is a member of the ginger family (*Zingiberaceae*), it is the principal curcuminoid, and chemically, curcumin is a diarylheptanoid. Recent research has shown that curcumin has a whole variety of medicinal activities: it modulates various signaling molecules, including inflammatory molecules, transcription factors, kinases, carrier proteins, cell survival proteins, drug resistance proteins, adhesion molecules, growth factors, receptors, cell-cycle regulatory proteins, chemokines, DNA, RNA, and metal ions (Gupta SC et al. 2012).

Luteolin (3',4',5,7-tetrahydroxyflavone), a flavonoid extracted from *Thymus vulgaris*, inhibited PKC ϵ and SRC kinase activity. In SKH-1 hairless mice, luteolin suppressed tumor incidence, multiplicity, and overall tumor size. Analysis of the skin by immunohistochemistry and immunoblotting showed that luteolin-treated groups had a substantial reduction in the levels of cyclooxygenase-2, tumor necrosis factor- α , and proliferating cell nuclear antigen compared with groups treated with only UVB. These data suggested that luteolin exerts potent chemopreventive activity against UVB-induced skin cancer mainly by targeting PKC ϵ and SRC (Byun S et al. 2010). Luteolin reduced the activity of catalase and superoxide dismutase, and the level of oxidative damage, and lipid peroxidation, in lung tissue, and reduced LPS-induced activation of MAPK and NF κ B pathways in mice (Kuo MY et al. 2011). Luteolin has also been demonstrated to inhibit lipopolysaccharide-induced tumor necrosis factor- α release and activation of the NF κ B pathway in macrophages. In adipocytes, luteolin markedly enhanced PPAR γ transcriptional activity in 3T3-L1 adipocytes, and luteolin-increased expression of adiponectin and leptin was blocked by GW9662, a PPAR γ antagonist. The data suggest that luteolin influences insulin action and production of adipokines/cytokines in adipocytes by activating the PPAR γ pathway (Ding L et al. 2010). Luteolin interfered with the regulation of estrogen signaling and cell cycle, consistent with the anti-estrogenic and anti-proliferative properties of luteolin in normal and malignant cells (Markaverich BM et al. 2011).

A third plant, aloe, was shown to reduce obesity-induced inflammation and the occurrence of metabolic disorders such as blood glucose and insulin resistance in obese mice. Obesity-induced inflammatory cytokine (IL-1b, IL-6, IL-12, TNF α) and chemokine (CX3CL1, CCL5) mRNA and protein were markedly decreased, as was macrophage infiltration and hepatic triglycerides by aloe. At the

same time, aloe down-regulated the mRNA and protein of PPAR γ /LXR α and 11 β -HSD1 (Shin E et al. 2011). Treatment with aloe decreased the expression of these adipogenic genes and repressed adipocyte differentiation. In addition, aloe suppressed the differentiation of human mesenchymal stem cells into adipocytes by downregulating PPAR γ and C/EBP α expressions indicating an anti-adipogenic effect (Subash-Babu P and Alshatwi AA 2012).

CONCLUSION

This transcriptomic analysis shows the induction of four major activities, as determined by the main functions of induced genes and their correlations. These are 1) the retinoic acid pathway, 2) proliferation and cell cycle control, 3) cell growth and remodeling as well as 4) protection against microorganisms, antioxidant properties, and ultraviolet light. The pathways and gene correlations concern proliferation and differentiation and thus favor rejuvenating and anti-apoptotic activities, and cell renewal. The protective mechanisms induced by the treatment cover a broad range of targets: antimicrobial, antioxidant, anti-allergic and UVB protection. Some of these activities mimic features described with other plant extracts. The active ingredients of aloe, curcumin and the compound luteolin share properties that can be found in essential sandalwood oil, which may result in sandalwood sharing some of the beneficial activities.

This analysis provides the gene expression basis for a range of valuable properties, some of which are under clinical investigation or confirms traditional medicinal uses. The absence of adverse effects and allergic potential underlines the usefulness to combine traditional medicine with a scientific approach to achieve targeted applications in skin care.

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TABLE**Table 1:** Typical composition of essential oils after a standardized extraction of sandalwood.

Name	%
Z- α -santalol	46,35
Z- β -santalol	19,11
Z-trans- α -bergamotol	5,66
Z-epi- β -santalol	3,91
Z- α -santalal	3,86
cis-lanceol	2,8
cis-nuciferol	1,78
E- β -santalol	1,63
spirosantalol	0,98
cyclosantalal	0,93
β -santalene	0,84
epi-cyclosantalal	0,58
epi- β -santalene	0,54
α -santalene	0,51
trans- α -santalol	0,33
α -bisabolol	0,18
α -curcumene	0,17
trans- α -bergamotene	0,1

Table 2: Mean fold-change induced by sandalwood oil in at least two out of three explants. Two concentrations were tested and results of 9h and 24h treatment are shown. Significant changes of expression are in **bold**.

GeneName	P1 (2%) 9h	SD	P3 (0,4%) 9h	SD	P1 (2%) 24h	SD	P3 (0,4%) 24h	SD
ABCC10	1,85	0,40	1,58	0,13	1,07	0,19	0,84	0,09
ADAMTS4	1,29	0,25	1,61	0,47	1,50	0,19	1,37	0,13
BCL6B	1,49	0,49	1,14	0,32	1,43	0,12	1,55	0,13
BOP1	1,40	0,06	1,07	0,08	1,05	0,12	1,01	0,08
CAPN2	1,20	0,23	1,08	0,22	1,34	0,06	1,10	0,15
CCND1	1,34	0,12	0,99	0,15	0,94	0,14	0,93	0,10
CRABP2	0,93	0,27	0,83	0,15	1,49	0,17	1,09	0,15
DDX3Y	1,56	0,11	1,37	0,16	1,13	0,13	0,90	0,15
DDX5	1,37	0,14	1,14	0,16	1,14	0,12	1,29	0,14
DDX6	1,29	0,06	1,10	0,15	1,02	0,13	0,91	0,12
DOK3	1,58	0,20	2,62	0,72	1,03	0,27	0,91	0,17
ELMO1	1,48	0,31	1,81	0,58	0,88	0,23	1,37	0,18
FGFR4	1,42	0,16	1,79	0,40	0,96	0,16	1,03	0,06
GJB2	0,91	0,49	1,57	0,15	1,40	0,18	0,93	0,28
IDO1	0,87	0,19	0,85	0,17	1,33	0,12	1,35	0,16
ITGAM	0,94	0,34	0,93	0,25	1,38	0,11	1,04	0,11
KRT8	1,49	0,22	1,08	0,18	0,91	0,18	0,83	0,17
LOXL2	1,01	0,10	1,41	0,30	1,58	0,24	1,43	0,17
NAMPT	0,93	0,11	1,35	0,09	1,32	0,06	1,12	0,15
NES	1,64	0,61	1,29	0,48	1,37	0,11	1,04	0,30
ODC1	1,39	0,03	1,01	0,25	1,45	0,32	1,07	0,31
PCNA	1,46	0,18	1,01	0,13	1,02	0,12	0,91	0,10
PLIN2	0,83	0,17	0,89	0,11	1,35	0,12	1,11	0,16
PPARG	1,18	0,16	0,99	0,29	1,57	0,19	1,20	0,31
PPP1R3F	1,42	0,11	1,65	0,32	0,98	0,21	1,06	0,27
RAB1B	2,32	0,03	1,74	0,35	0,97	0,10	0,82	0,05
SRC	1,38	0,12	1,35	0,10	0,96	0,13	0,96	0,15
SULT1A2	1,00	0,17	1,33	0,26	1,34	0,13	1,41	0,14

FIGURES

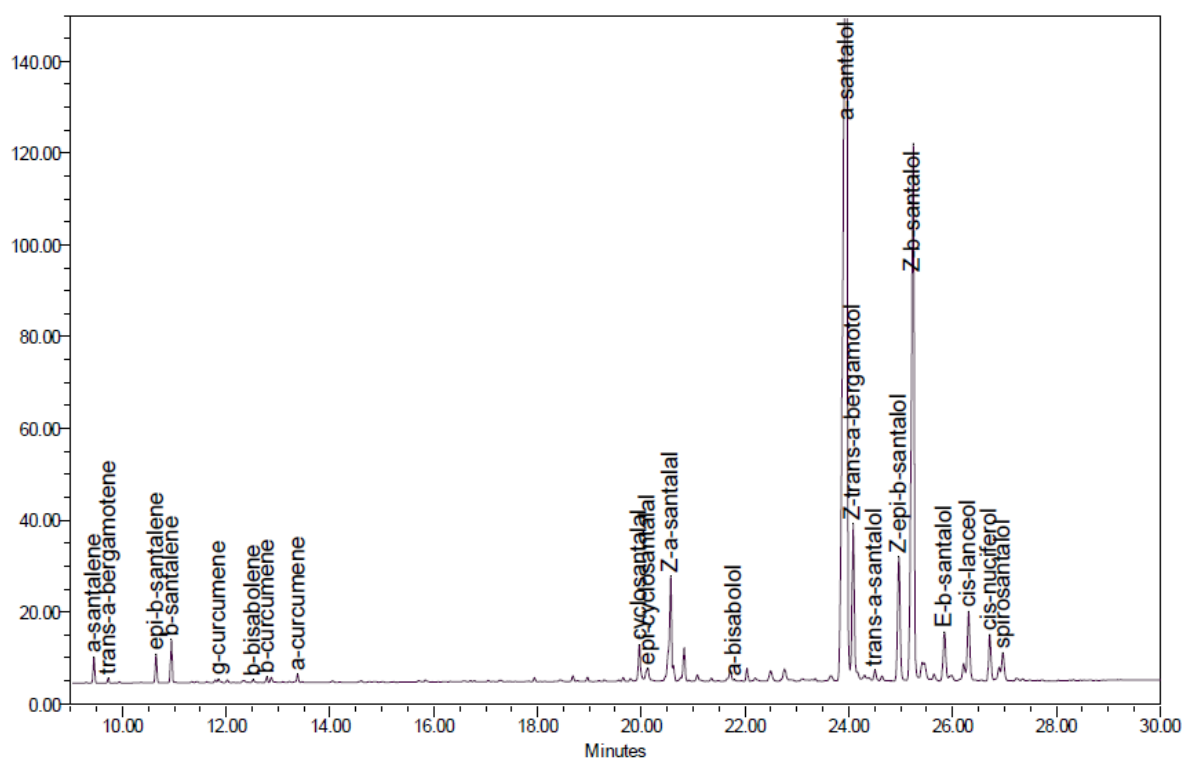


Figure 1: Typical GC chromatogram of East Indian *Santalum album* L. essential oil. It complies with the international standard ISO 3518/2002 for the quality of sandalwood oil.

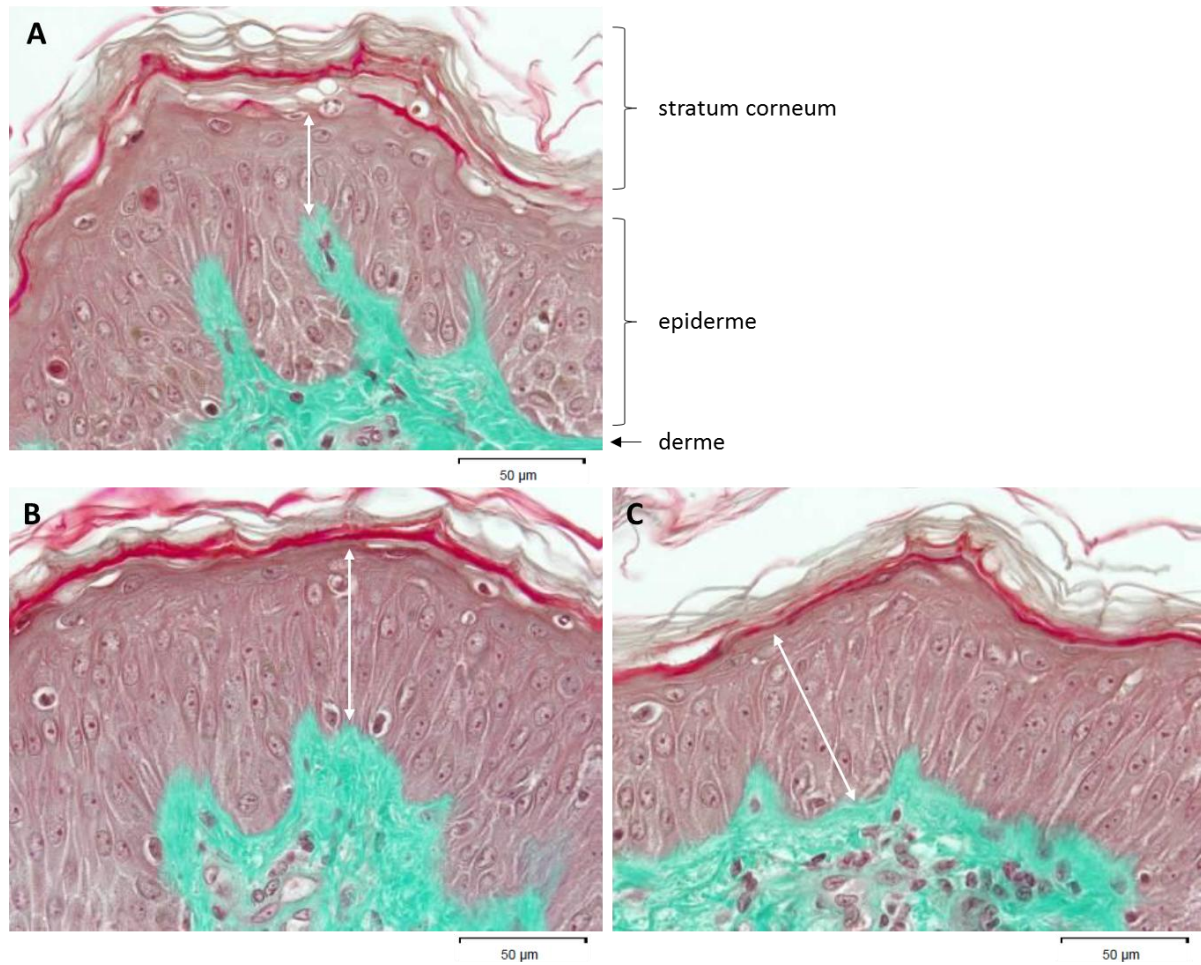


Figure 2: Morphological comparison after staining of paraffinized sections according to Masson's trichrome, Goldner variant. (A) Explant control after 8 days in culture, (B) explant treated with the excipient almond oil, and (C) essential sandalwood oil at 2% on day 8. White arrows indicate the thickness of the epithelial layer.

Legend for **Figure 3**

