PHARMACOLOGICAL PROPERTIES OF EISO

(EAST INDIAN SANDALWOOD OIL)

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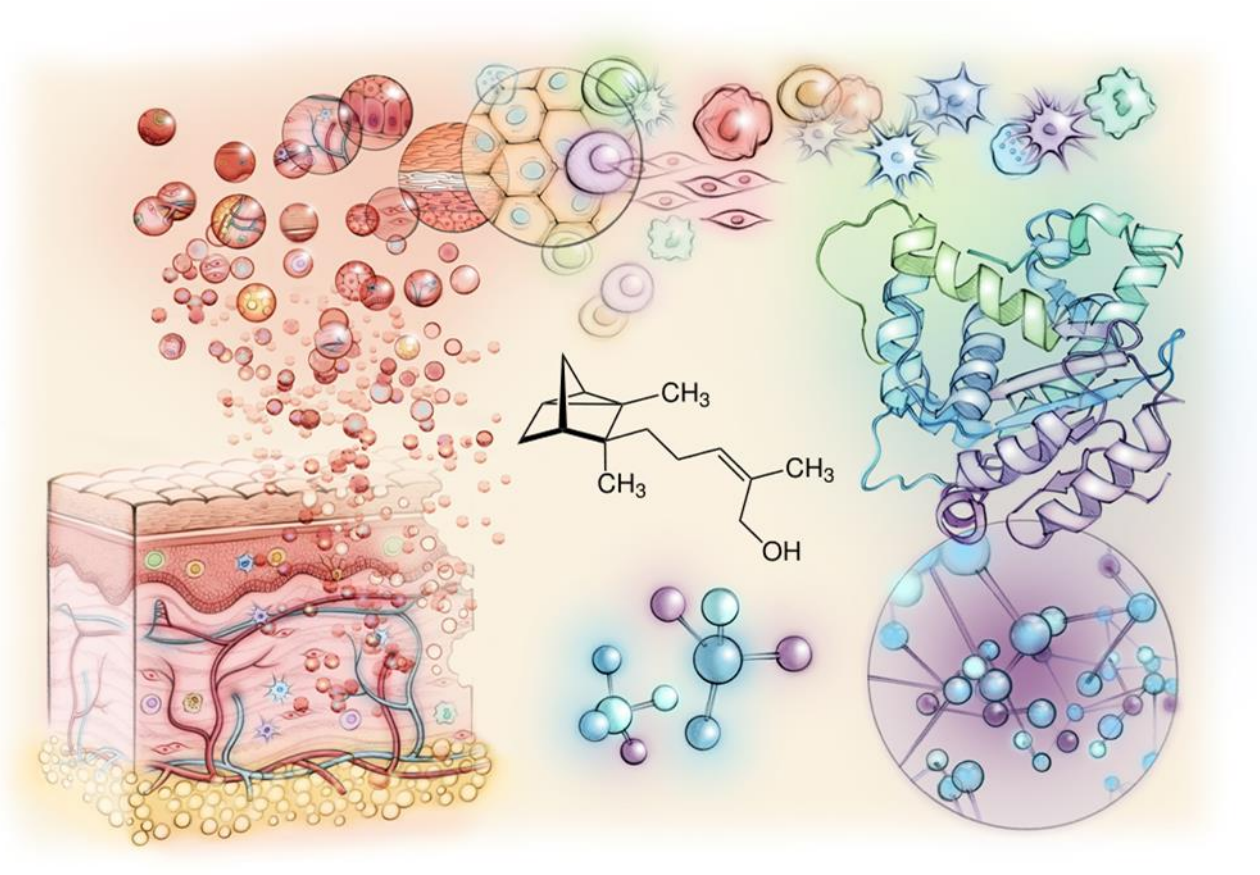
APPLICATIONS IN DERMATOLOGY

WITH FOCUS ON SKIN HEALTH



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SUMMARY TABLE

EXECUTIVE SUMMARY

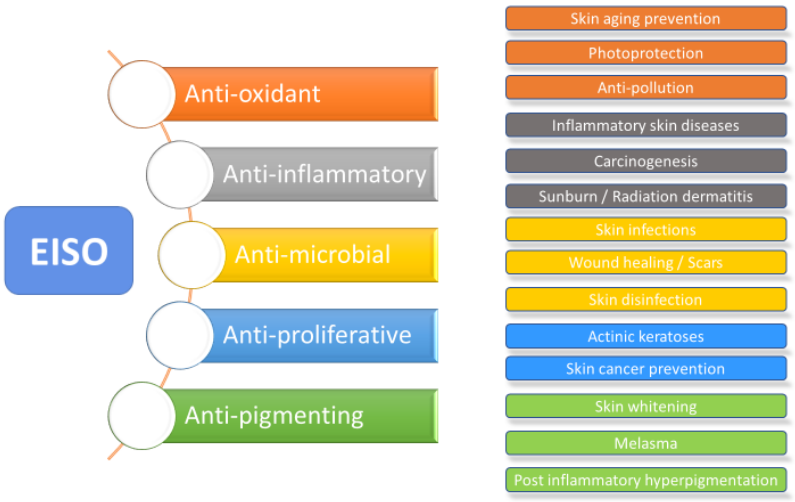
Sandalwood is one of the longest-established medicinal aromatic plants and has had a continuous history of human use for at least 3,000 years. As early as 900 BCE, powdered sandalwood was used in personal care, both in bathing and as a paste applied to the skin. Its use is described across the globe and civilizations by Ancient Egyptians, Tibetans, Ayurvedic doctors and Traditional Chinese Medicine experts. Interestingly, in the nineteenth century, Indian sandalwood oil was added to pharmacopoeias of Britain, Germany and Belgium.

The therapeutic value of Sandalwood oil (SO) is related to its high content in natural sesquiterpenes, namely, alpha-santalol (55% of the oil) and beta-santalol (20% of the oil). In medicine its use is linked to 5 main pharmacological properties: SO, acts thanks to antioxidant, anti-inflammatory, antimicrobial, anti-proliferative and anti-pigmenting properties.

This polypharmacological profile is particularly relevant for the skin, our interface with the environment and primary defense line against external stressors such as radiations, temperature variations or microbial threats. To ensure this protection, skin is not only a mechanical barrier made of cornified dead cell envelopes, but a complex living organ where multiple biological processes take place.

This document reviews the 5 key properties of East Indian Sandalwood Oil (EISO), describes their implication in skin homeostasis and skin diseases, reports the data generated with Sandalwood oil or EISO and recommends on the potential uses and positioning of this ingredient in cosmetics or OTC products.

All indications are not supported with the same level of evidence and some of them would certainly need further research work or clinical investigation. Nonetheless, the body of data available confirms that EISO is a gift from Nature and a great protector of skin health. Forteen areas of interest were identified as summarized in the figure below:



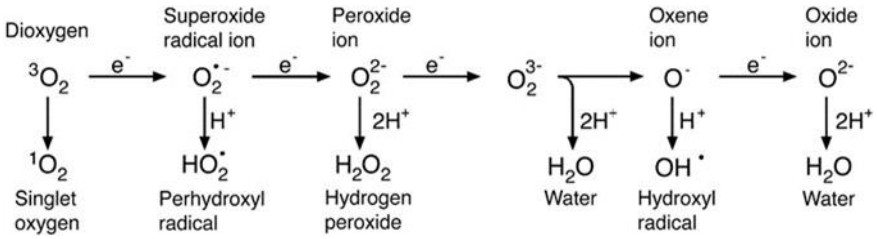
A. Anti-oxidant properties of EISO

Rationale

The skin cells continuously produce, through cellular respiration, metabolic processes or under external aggressions, highly reactive molecules, generally called free radicals. Free radicals are molecules with an unpaired electron like reactive oxygen species (ROS), peroxides, superoxide anion, hydroxyl radical and singlet oxygen. These molecules are immediately neutralized by enzymatic (glutathione peroxidase, catalase and superoxide dismutase) and non-enzymatic (estradiol, melatonin, vitamin E and C) systems in a physiological and dynamic balance.

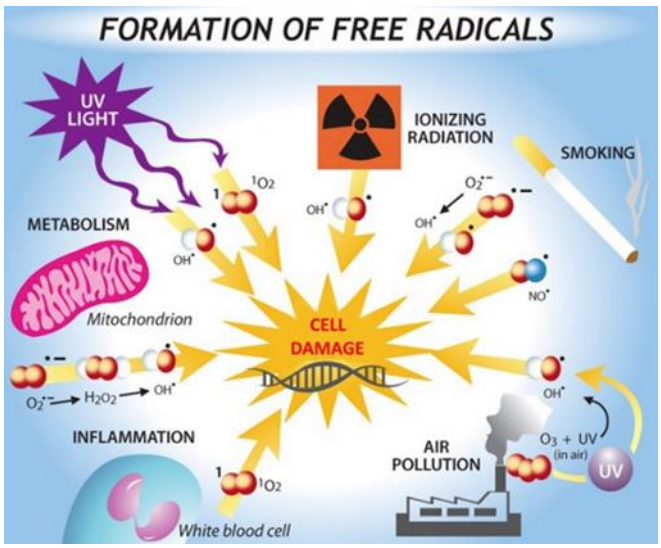
Generation of different ROS by energy transfer or sequential univalent reduction of ground-state triplet oxygen

From: Rusty Rodriguez, and Regina Redman PNAS 2005;102:9:3175-3176



Oxidative stress is defined as an imbalance between the formation of oxidative free radicals and the antioxidant defense capacity of cells of the body. In this situation various cellular structures may suffer structural modifications, triggering or worsening skin conditions or diseases: interaction with nucleic acids (both in the mitochondria and in the nucleus) results in mutations that predispose to DNA strand breaks; oxidation of lipids by ROS can damage cellular structures, for example the phospholipid cellular membranes, and result in premature cell death; interaction with proteins may lead to oxidation of individual amino acids thereby altering their structure and impairing enzymatic function of the protein.

By impairing the tight redox balance, free radical excess is clearly implicated in the etiopathogenesis of various dermatoses, as well as in the aging process and in the onset of cutaneous neoplasias.



Indications of interest

Photoprotection and photocarcinogenesis.

Use of antioxidants in the prevention and repair of Ultraviolet as well as Visible light (responsible for 50% of the total oxidative stress caused by sunlight) photodamage is widely studied, being the most known and used indication. Beside the extrinsic aging, the intrinsic aging process, shows progressive damage to mitochondrial DNA, with again an increase in ROS production, which causes cell aging. The association of antioxidant molecules, from vitamins to phytoextracts, in photoprotectors and moisturizers with appeal against aging, is thus very frequent. Antioxidants are complementary to photoprotection with sun filters, and this combination is currently the most adequate form of photocarcinogenesis prevention.

Aging

Another external skin stressor is airborne pollution with pollutants reaching the superficial and deeper skin layers by transcutaneous and systemic routes after pollutant inhalation or ingestion. The main outdoor air pollutants derive from gaseous compounds (nitrogen dioxide [NO2], sulphur dioxide [SO2], carbon monoxide [CO]), particulate matter (PM) and heavy metals. Other classes of air pollutants are persistent organic compounds (POPs), semi-volatile compounds (SVOCs) and polyaromatic hydrocarbons (present in cigarette smoke). Interestingly, some of those pollutants become even more toxic in the presence of UV radiation. Pollutants may activate cutaneous metabolism and inflammatory pathways and induce oxidative stress by lowering the levels of antioxidants in particular.

A 'free radical theory of greying' has also been proposed to characterize the ageing process taking place in hair. As hair ages, a reduction in antioxidant enzymes, such as catalase, and an increase in ROS, particularly H2O2 has been found suggesting redox imbalance and an increase in oxidative stress, ultimately reducing melanin synthesis and causing loss of hair color.

Others

Eventually, other inflammatory skin conditions demonstrate redox imbalance and significant consumption of their antioxidant systems in local cells: atopic dermatitis, psoriasis, vitiligo, lichen planus, acne vulgaris, seborrheic dermatitis, rosacea, alopecia areata or burned skin.

Data available with Sandalwood oil

A Santalum album extract demonstrated significant anti-oxidant properties in two independent studies (Luo 2007, Kamal 2012) using a DPPH model (DPPH (2,2-diphenyl-1-picrylhydrazyl) is a free radical, stable at room temperature, which produces a purple colour solution in methanol). It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured methanol solutions). Interestingly, the S album extract presented the second-best antiradical efficiency in the 20 plant extracts tested in the second study and its anti-oxidant activity was further improved by adding Vit A, E and C to the mixture.

The antioxidant activities of EISO and santalol isomers (purified from EISO by sequential column chromatography on silica gel followed by supercritical fluid chromatography) were measured by the

same DPPH radical quenching experiment. The antioxidant activity of EISO, santalol isomers and reference antioxidant compound (vitamin c) was tested in a cell free system. The results of this DPPH assay confirmed that EISO, alpha- and beta- santalol possess noticeable antioxidant activity as compared to vitamin C in vitro. The magnitude of DPPH reduction (IC50 DPPH) by EISO, alpha- and beta- santalol was observed at a concentration of 65.11 ± 0.41 µg/mL, 42.34 ± 0.71 µM and 34.87 ± 0.97 µM, respectively, when the reduction was 3.26 µg/mL or 18.51 µM for vitamin C. Beta-Santalol exhibited higher antioxidant activity than alpha-santalol, possibly because of the presence of unsaturated active alkene group in the second position over the eight member ring.

Data generated by Quintis ***https://doi.org/10.3390/cosmetics8020053***

- A Cellular Antioxidant Activity Assay (CAA) using AAPH (2,2'-Azobis(2-amidinopropane) dihydrochloride), as a free radicals initiator was performed to evaluate the antioxidant potential of Sandalwood oil comparatively to a reference control (Quercetin) in a cellular based assay using the HaCaT cell line. Sandal Wood oil showed antioxidant potential at concentrations as low as 0.025%. The antioxidant potency of Sandal wood oil was equivalent to what was observed with the highest concentrations of Quercetin tested. Specifically, at a concentration of 0.2%, Sandal wood oil has an antioxidant activity which is as potent as the antioxidant activity of the positive control Quercetin at 0.0075%.

- An experiment evaluated the Sandalwood oil ability to quench Reactive Oxygen Species (ROS) after exposure to external stressors. Three different environmental stressors were used in this study namely Blue light 412 nm, Blue light 450 nm and cigarette smoke. Both wavelengths of blue light were tested at 3 different doses: 200 mJ/cm$_2$, 500 mJ/cm$_2$ and 1000 mJ/cm$_2$ and cigarette smoke was generated by 3 cigarettes. The oxidative stress (monitoring of level of ROS) was examined through the DCFH-DA method using the HaCaT cell line and comparatively to two positive controls, tocopherol (Vitamin E) and quercetin. A dose dependent protection against ROS induction could be observed with Sandalwood oil. This effect was superior to the effect of tocopherol and quercetin in the two Blue light experiments and equivalent to the tocopherol effect in the cigarette smoke experiment (in this experiment quercetin did not show any protective effect).

- The efficacy of Sandalwood Album Oil to inhibit the aging effect of pollution (ozone (1.6 ppm in 20 mn) and cigarette smoke (30 minutes exposure, 3 cigarettes)) was assessed on full skin explants (obtained from an anonymous donor following a mastopexy) by monitoring the levels of metalloproteinase-1 (MMP-1) through an ELISA assay. Matrix metalloproteinase-1 (MMP-1) is a zinc and calcium dependent endopeptidase that is synthesized and released from both dermal fibroblast and keratinocytes. An elevated expression of its activity has been implicated with a degradation of the extracellular matrix and lead to premature photoaging of the human skin as well as skin cancer. Sandal Wood Oil significantly inhibited the expression of MMP-1 compared to untreated samples with a reduction by 88% in the ozone assay and a reduction by 70% in the cigarette smoke experiment.

These three studies confirm the property of Indian sandalwood oil as a potent ingredient against oxidative stress and its protective effect against the detrimental consequences of environmental stressors such as blue light and pollution on human skin. (Francois-Newton V, 2021). These in vitro findings were recently confirmed in a clinical trial enrolling 22 subjects (Kolanthen,2022) **https://doi.org/10.3390/cosmetics9020035**.

Conclusion

Oxydative stress is THE skin homeostasis perturbator. It plays a major role in skin aging, photocarcinogenesis or melasma and contributes to chronic inflammation in many other skin diseases.

Sandalwood oil and its main component alpha-santalol have been shown to be anti-oxidants with potential superiority over quercetin and tocopherol and possible additive effects to other anti-oxidants like Vit A, E or C.

Potential positioning: photoprotection, sunburns, aging (including greying hair), melasma and pigmentary disorders, mild inflammatory skin diseases (rosacea, seborrheic dermatitis)

Positioning and claims

By mechanism: Protects against free radicals, Protection of DNA, proteins and lipids from oxidative damage, help maintaining skin health/homeostasis, revitalize

By effect: decreases the deleterious effects of UV light and pollution, treats and prevents fine wrinkles, age spots, and rough skin caused by sun exposure or airborne pollution, reduce signs of sun damage and aging , rescue skin, youth activator, rejuvenating or invigorating agent, replenishes or firms skin

By indication : Anti-aging, Photodamage prevention, Anti-pollution, treatment of sunburns

B. Anti-inflammatory properties of EISO

Rationale

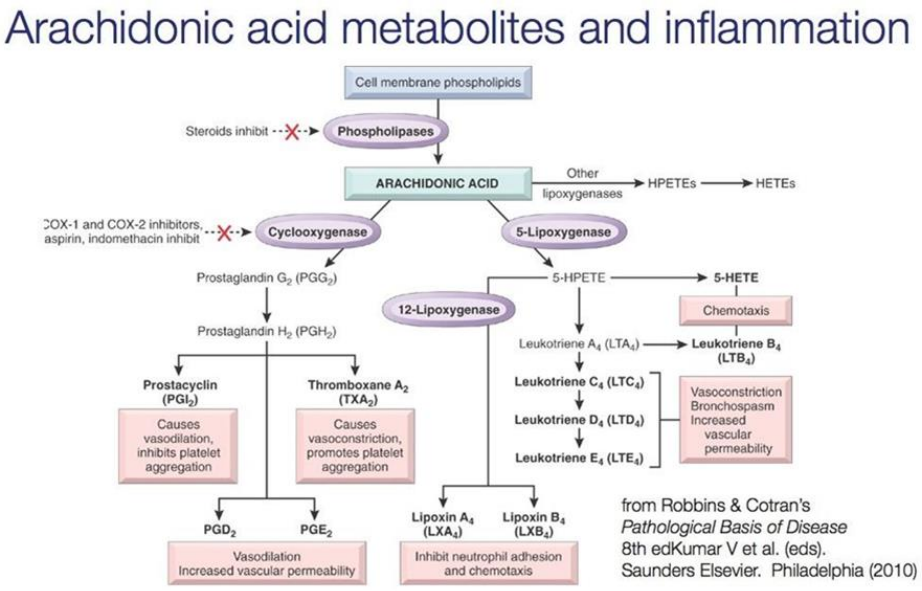
Sandalwood oil is routinely used in India as an Ayurvedic medicine for inflammatory and eruptive skin diseases. These anti-inflammatory effects are the result of a polypharmacological profile. Four pathways impacted by Santalols, the main components of Sandalwood oil, are identified: 5-lipoxygenase, COX2, PDE4 and 11Beta HSD1.

1. <u>5-lipoxygenase</u>

Lipoxygenases (LOs) catalyze the oxygenation of polyunsaturated fatty acids such as arachidonic acid and linoleic acid. The oxygenated lipids initiate subsequent biological reactions, activate cellular signaling mechanisms through specific cell surface receptors, or are further metabolized into potent lipid mediators such as Leukotrienes and particularly LTB4 and LTC4. Those molecules have prominent functions in pathophysiology as they act as neutrophil activators and attractants as well as strong immune response activators. They are recognized as outstanding mediators involved in persistent and chronic inflammatory skin diseases and are thus connected to numerous inflammatory disorders including Acne (Zouboulis 2009), Psoriasis (Iversen 1997), Atopic dermatitis, Wound healing, Systemic Sclerosis or Graft-versus-host disease.

2. <u>Cyclooxygenase 2</u>

Cyclooxygenase (which exists under two isoforms, COX-1 and COX-2) is responsible for the conversion of arachidonic acid to Prostaglandin H2 which is the precursor for the biosynthesis of Prostaglandins E2 and I2, two important inflammatory mediators. PGE-2 exerts its effects on target cells by binding to and activating one (or more) of four PGE-2 receptors, classified as EP1–EP4. Most non-steroidal anti-inflammatory drugs (NSAIDS) are COX inhibitors.

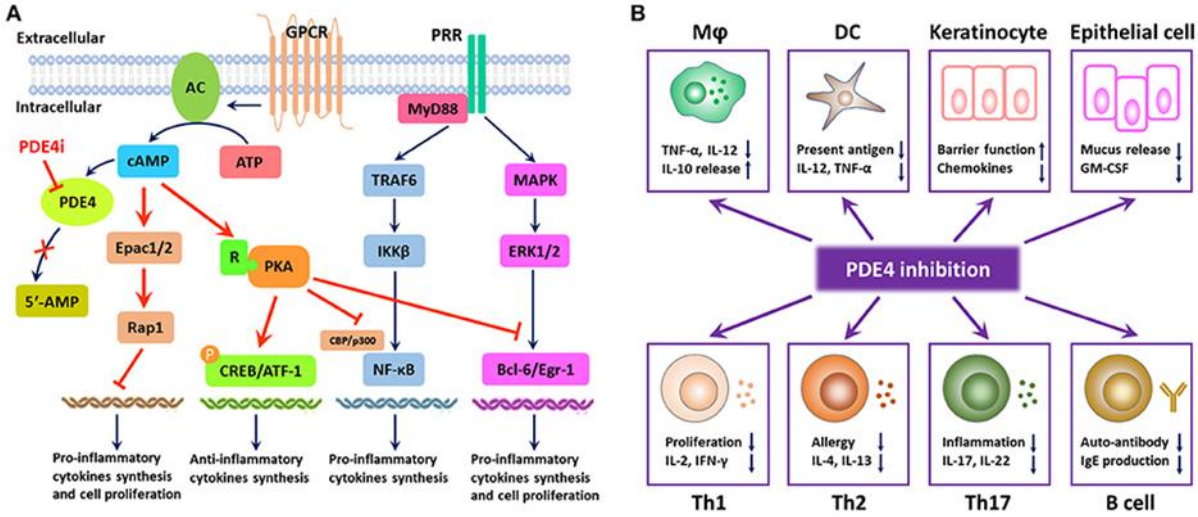


3. Phosphodiesterase 4

Phosphodiesterase (PDE) 4 is a superfamily of enzymes that catalyze the hydrolysis of cyclic adenosine 3',5'-monophosphate (cAMP), an intracellular second messenger and regulator of a wide array of genes and proteins. Increased levels of intracellular cAMP lead to activation of genes but also to inhibition of nuclear factor-kappa B, involved in pro-inflammatory responses. By increasing cAMP levels, PDE4 inhibitors reduce production of pro-inflammatory TNFα, IFNγ, and IL-17 and increase production of anti-inflammatory IL-10. Among PDE4 inhibitors, apremilast, roflumilast, and crisabolore have been approved for the treatment of psoriasis and PsA, chronic obstructive pulmonary disease, and atopic dermatitis, respectively. Other suggested indications in dermatology include alopecia areata, lupus erythematosus, sclerosis, lichen planus, hidradenitis suppurativa, rosacea or cutaneous sarcoidosis (Maloney 2019).

Phosphodiesterase 4 Inhibition

https://www.frontiersin.org/article/10.3389/fphar.2018.01048

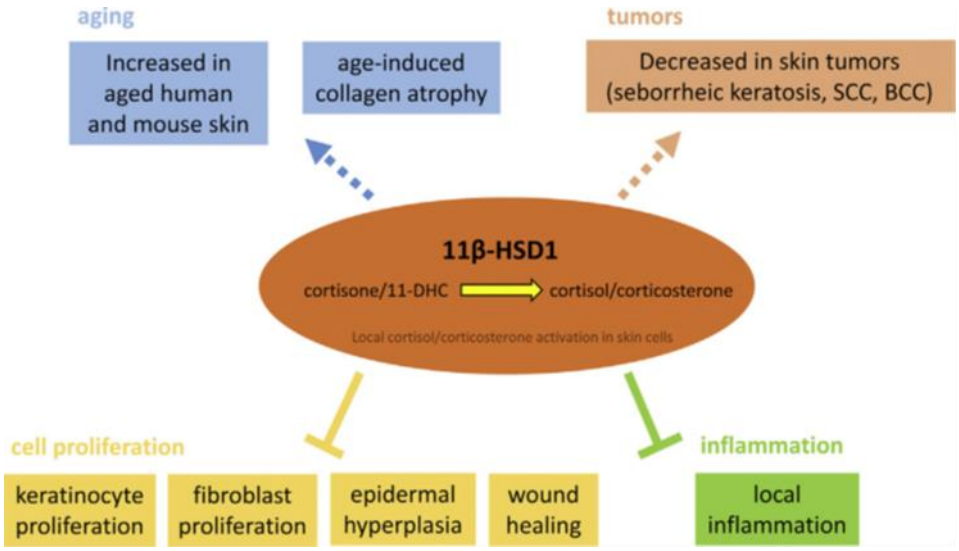


In several cell types such as monocytes, T cells, neutrophils, and endothelial cells, cyclic Adenosine Monophosphate (cAMP) is degraded to AMP mainly by PDE4. PDE4 inhibition increases intracellular cAMP levels and determines the activation of Protein Kinase A (PKA). PKA activation induces the phosphorylation of transcription factors such as CREB, that in turn binds the promoters of genes encoding from various anti-inflammatory (IL-10) cytokines.

4. 11 Beta hydroxysteroid dehydrogenase 1 (11β-HSD1)

Human skin has the capacity to produce glucocorticoids, androgens and estrogens from de novo synthesis of cholesterol. Besides, the local concentration of cortisol is controlled by 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes, catalyzing the interconversion of active cortisol and inactive cortisone. 11β-HSD1 is a bidirectional enzyme utilizing cofactor NADPH and acts in vivo predominantly as an oxoreductase converting cortisone to cortisol. It is widely expressed and in skin it has been detected in keratinocytes, dermal fibroblasts and the outer root sheath of hair follicles. It has been suggested that 11β-HSD1 may suppress UVB-induced inflammation via inhibition of NF-κB activation. Also, 11β-HSD1 negatively regulates cutaneous wound healing by modulating proliferation of keratinocytes and fibroblasts. Eventually the expression of 11β-HSD1 is decreased in benign and malignant tumours of the skin, such as squamous cell carcinoma, basal cell carcinoma, and seborrhoeic keratosis, indicating the association of 11β-HSD1 with cell proliferation (Itoi-Ochi 2016).

Biology of 11-Beta-HSD1



Indications of interest

1. <u>5-lipoxygenase inhibition</u>

ACNE: Leukotriene B4 (LTB4) is considered to be a major player in the development of tissue inflammation and enzymes involved in the biosynthesis of this molecule are activated in sebaceous glands of acne lesions: 5-LO shows a stronger expression in acne lesions than in normal skin or in uninvolved skin of acne patients. Its activation results, among other effects, in induced IL-6 and IL-8 expression in human sebocytes. Systemic treatment of 10 acne patients with a 5-LO inhibitor (Zileuton) reduces the inflammatory lesion count with a mean reduction in inflammatory lesions of 71% at 12 weeks. Interestingly a decrease in the synthesis of sebum lipids was also shown with a mean reduction of 65% at 12 weeks (Zouboulis 2009). Such effect could be useful in the management of skin conditions related or associated with sebum hyperproduction such as oily skin or seborrheic dermatitis.

PSORIASIS: it was suggested that 5-LO inhibitors could be beneficial in psoriasis because neutrophil activation is a prominent feature of the disease and since leukotriene-B4 is present in psoriasis lesions. In vitro LTB4 is a potent chemoattractant for leukocytes, and it increases DNA synthesis in human cultured keratinocytes. Intradermal injection of LTB4 into human skin in vivo results in a wheal and flare reaction, and topical application produces intraepidermal microabscesses and induces hyperproliferation as seen in psoriasis lesions. Results of clinical trials addressing the efficiency of leukotriene inhibition to treat psoriasis have however been inconclusive with some trials showing significant clinical improvement (Black 1990) and others finding no significant effects (van de Kerkhof 1996).

ATOPIC DERMATITIS: High levels of Leukotrienes B4 and C4 are found in skin lesions of eczema (Hua 2006). In small clinical and case studies, montelukast was found to be a safe and effective alternative or steroid-sparing therapy in the management of patients with atopic dermatitis (Nettis 2002).

WOUND HEALING: The cutaneous repair is a complex process that involves inflammation, tissue formation and remodeling. In the initial inflammatory and cellular reactions that follow injury, neutrophils are the first cells recruited to the damaged site. In addition to their defense functions, these cells are also an important source of growth factors and cytokines, which initiate the proliferative phase of wound healing by contributing to the proliferation or migration of keratinocytes and dermal fibroblasts to the lesion. The absence of 5-lipoxygenase (5-LO) leads to faster skin wounds closure and this result is related to both a restrained inflammation and a higher collagen production (Guimarães 2018). 5-LO is consequently a potential target for the treatment of cutaneous healing in cases of deficient or unresolved repair, such as in large and threatening lesions, burnt patients, decubitus or diabetic ulcers.

HYPERTROPHIC SCARS AND KELOIDS: Keloids and hypertrophic scars (HS) are disorders characterized by excessive accumulation of extracellular matrix produced by fibroblasts. Clinically, HS do not extend the lesion's border, often regress, and have a better prognosis than the keloids. Keloids have less cellularity and thick collagen bundles with irregular patterns, whereas the HS have more fibroblast proliferation and collagen fibers in nodules parallel to the epidermis. The inflammatory phase of the healing may be related to the formation of these pathologic scars. The derivatives of arachidonic acid, mainly prostaglandins and leukotrienes, play a fundamental role in this process (Henry 2003).

SKIN CANCER: Multiple evidence suggests that 5-LO-derived products play a role in skin cancer development. This evidence is based on the observation of aberrant LO expression and overshooting product formation as well as the cancer preventive activity of LO-inhibitors. Orally administered TMK866, a 5-LO inhibitor was shown to reduce tumor formation induced by 7,12-dimethylbenzanthracene (DMBA)/TPA or benzopyrene alone. Interestingly, combined inhibitors of 5-LO and COX2 were reported to synergistically reduce tumor growth of human squamous carcinoma cells inoculated in mice.

2. <u>COX2 inhibition</u>

SKIN AGING: Both UVA and UVB radiation stimulate the production of a variety of inflammatory mediators from keratinocytes, fibroblasts and infiltrating immune cells, including TNF-alpha, Il-1, IL-8 and PGE-2. PGE-2 plays a key role in the development of skin damage associated with both intrinsic (chronological) and extrinsic aging (primarily photoaging) by (Fuller 2019):

- Increasing the MMP production by keratinocytes, fibroblasts and immune cells, which results in the degradation of collagen and elastin
- Increasing the production of MCP-1 a chemokine involved in neutrophil and monocyte influx into the skin, resulting in the production of ROS, MMPs, and other inflammatory mediators that damage the dermal matrix
- Decreasing collagen I and III and fibronectin mRNA and protein production
- Increasing fibroblast senescence (cells are still viable, but they will not proliferate, and their gene expression pattern is altered with reduced capacity to make collagen but an increased capacity to make and secrete MMPs).

HYPERTROPHIC SCARS AND KELOIDS: see page 10

SKIN CANCER: with the possible exception of the EP3 receptor, all PGE-2 receptors have been found to contribute to the development and progression of skin cancer. The level of the EP1 receptor increases in keratinocytes after exposure of skin to UVR, and several studies have shown that the EP1 receptor is involved in UVR induced skin tumor promotion. Blocking EP1 receptor activity by pharmacological antagonists prevents UVR induced tumor formation in animal models. The EP2 receptor is also linked to tumor promotion, since, in animal models, EP2 knockout mice were resistant to chemically induced skin cancer. EP4 has been shown to be a tumor promoting receptor in skin, and in particular, can increase melanoma proliferation and metastasis (Fuller 2019). A topical formulation containing the COX inhibitor, diclofenac (it is non-selective for COX-1 and COX-2), and sold under the trade name of Solaraze®, is approved by the FDA for the treatment of Actinic Keratoses a lesion that is classified as an in-situ skin cancer by some authors.

SUNBURN: The acute UVB-induced inflammatory response in the skin is characterized by the rapid induction of COX-2 gene expression with the subsequent production of prostaglandins, including PGE2, resulting in dermal edema and the infiltration of activated neutrophils into the dermis. Inhibiting the infiltration of and activation of dermal neutrophils and blocking the activity of the COX enzymes prevents the cellular damage resulting from the production of reactive oxygen intermediates that may induce misreplication, misrepair and ultimately neoplastic development. This inhibition may have the ultimate effect of decreasing the number of apoptotic cells within the epidermis.

PIGMENTATION DISORDERS: Although there are a number of UVR-induced hormone-like factors, including MSH, ACTH, and Endothelin-1, that can stimulate melanin production in melanocytes, PGE-2 is one of the most potent stimulators of pigmentation in human melanocytes. In addition, prostaglandin were shown to modulate melanocyte dendricity, proliferation and tyrosinase expression. Thus, the increase in the synthesis of PGE-2 and its secretion from keratinocytes, fibroblasts and melanocytes themselves as a result of sun exposure or inflammation, plays a significant role in the formation of hyperpigmentation, solar lentigines (“age spots”) and suggests that COX-2 inhibitors can potentially be used in whitening and for pigmentary disorders like melasma and post-inflammatory pigmentation (Kim 2012).

ROSACEA: Oral and topical non-steroidal anti-inflammatory agents such as piroxicam and diclofenac may reduce the discomfort and redness of rosacea. A clinical study compared the efficacy of metronidazole 0.75% gel to piroxicam 0.5% gel 40 patients with moderate to severe rosacea. Treatments were applied twice daily for 15 weeks. There was a statistically significant difference in the number of papules, pustules and total lesion counts for both drugs between baseline and each of the following visits. After 3 weeks, 56-59% improvement in papules and 66-73% improvement in pustules were observed for piroxicam and metronidazole respectively. This effect was more pronounced after 6 weeks of treatment and reached its maximum after 9 weeks of treatment 78-79% improvement in papules and 79-92% improvement in pustules. Both treatments were effective in reducing erythema starting from visit 2 (Patent WO2002074290A2).

3. Phosphodiesterase 4 inhibition

PSORIASIS: Apremilast, an orally administered small molecule inhibitor of phosphodiesterase 4, has been aproved by the US Food and Drug Administration for the management of moderate to severe plaque psoriasis in 2014. The efficacy and safety of apremilast were established through well-conducted clinical trials which showed superior efficacy over placebo demonstrating the relevance of this mechanism of action in the disease. It is however interesting to note that this result was achieved with a systemic route when all topical developments targeting PDE4 in psoriasis failed or were stopped, indicating a limited benefit. In addition there is no clinical trial testing a PDE4 compound by topical route on Clinicaltrial.gov website.

ATOPIC DERMATITIS: AD is believed to result primarily from overexpression of the T helper (Th) 2 cytokine axis; however, Th22, Th17 and Th1 cytokine pathways may also play a role, especially in Asians in whom the pathophysiology of AD could be slightly different. With regard to AD, PDE4 is found in immune and inflammatory cells such as basophils, mast cells, eosinophils, B and T lymphocytes, monocytes, macrophages, neutrophils and endothelial cells. Increased cAMP-PDE activity has been reported in freshly isolated mononuclear lymphocyte preparations as well as in dermal fibroblasts from patients with AD. Interestingly, increased PDE activity in atopic monocytes was associated with increased prostaglandin E2 production, resulting in increased IL-6 and increased IL10, with both contributing to Th1/Th2 imbalance. Increases in cAMP levels as a result of PDE4 inhibition in cellular studies of atopic leukocytes have been associated with the suppression of proinflammatory molecules such as IL-4 and prostaglandin E2, suggesting that inhibition of PDE4 may decrease the inflammatory processes associated with AD (Guttman-Yassky 2019). GSK is developing an oral PDE4 inhibitor (GW842470X) and Astra-Zeneka is developing topical roflumilast cream in AD.

ALOPECIA AREATA: Alopecia areata (AA) is a prevalent autoimmune disease causing loss of hair, with approximately 2% of the population suffering from the condition during their lifetime. The first cytokines to be identified as AA-related were IL-1 and Th1/interferon (IFN)-c but other immune pathways were also shown to be upregulated including PDE4 which is highly increased in human AA scalp lesions, representing a potential therapeutic target

ROSACEA: In an investigator-initiated, open-label pilot study, 10 patients with moderate to severe inflammatory rosacea were administered apremilast, 20mg orally twice daily, for 12 weeks. When baseline ratings were compared with those at end of treatment, measures that reached statistical significance were Physician Overall Erythema Severity and non transient erythema. Papule and pustule counts were not affected by treatment, neither as chromametry (Thompson 2014).

HIDRADENITIS SUPPURATIVA: Hidradenitis suppurativa, also called Acne inversa, is an inflammatory skin disease that affects apocrine gland-bearing skin in the axillae, in the groin, and under the breasts. It is characterised by recurrent boil-like nodules and abscesses that culminate in pus-like discharge, difficult-to-heal open wounds (sinuses) and scarring. Hidradenitis suppurativa has a significant psychological impact, and many patients suffer from impairment of body image, depression and anxiety. A study assessed the efficacy of the oral phosphodiesterase 4 inhibitor apremilast (30 mg, twice a day) in the management of 6 patients with moderate to severe hidradenitis suppurativa (Hurley stage II-III) who had responded poorly to other treatments. Patients reported a mean standard deviation pain VAS score of 7.17 before treatment and 2.00 after therapy, indicating a significant decrease in pain (Weber 2017).

4. 11 Beta hydroxysteroiddehydrogenase 1 (HSD1) activation

SUNBURN: Activation of corticol in keratinocytes is considered important for the suppression of local inflammation via the suppression of NF-κB activation following UVB radiation and constitutes a local first-line defense to protect the skin from various stressors, such as physical injury and psychological stress.

SKIN CANCER: In benign and malignant skin tumors, the expression of 11b-HSD1 is decreased in basal cell carcinoma and squamous cell carcinoma.

PSORIASIS: The expression of 11b-HSD1 is decreased in psoriasis vulgaris epidermis as evaluated by immunohistochemistry and western blotting. In addition, 11b-HSD1 expression is lower in lesional psoriasis vulgaris skin than in marginal skin.

Data available with Sandalwood oil

1. <u>In vitro studies</u>

5-lipoxygenase inhibition

A first study assessed in vitro, the potential of 32 essential oils, 10 absolutes and 26 natural or nature-identical chemicals to inhibit the enzyme 5-LO, in comparison to nordihydroguaiaretic acid (NDGA) used as reference compound due to its widely reported strong inhibitory activity on this enzyme. In this assay Santalum spicatum was scored as a strong inhibitor when Santalum album was classified as a moderate inhibitor (Baylac 2003).

A second study tested 25 essential oils for their anti-oxidant and anti-lipoxygenase properties and confirmed the activity of Sandalwood oil at 5 µg/ml (inhibition by more than 70% of the lipoxygenase activity) (Wei 2010).

COX2 inhibition

An experiment addressed the anti-inflammatory effects of Sandalwood oil preparations on skin, using human epidermal keratinocyte/dermal fibroblast co-culture models with Lipopolysaccharide (LPS), used as an inflammatory stimulus, and Ibuprofen as an anti-inflammatory positive control. The similar ability of the Sandalwood oil preparations and Ibuprofen to suppress expression of the LPS stimulated chemokine and cytokines included in the antibody arrays suggests that the Sandalwood oil may have a similar mechanism of action, i.e. inhibition of COX and production of the resulting arachidonic acid metabolites. Santalols are the primary factors contributing to the anti-inflammatory properties reported and it was shown that β-santalol was as effective as α-santalol at suppressing LPS-stimulated proinflammatory events. Interestingly East Indian Sandalwood Oil (EISO) was more effective than Western Australian SO (WASO), presumably because of the higher concentrations of the α-santalol and β-santalol (Sharma 2014).

In another experiment, Sharma tested the efficacy of East Indian Sandalwood oil (EISO) and Ibuprofen (IB) on markers of epidermal differentiation, proliferation and inflammation by employing three dimensional models of reconstituted psoriatic human skin prepared from normal human epidermal keratinocytes and psoriatic fibroblasts harvested from psoriasis lesions. The reconstituted human skin

equivalents were treated in the absence or presence of EISO or IB for 2, 3 and 8 days and culture supernatants were analyzed for key indicators using ELISA (ENA-78, IL-6, IL-8, GM-CSF,MCP-1, IL-1β and IL-17). Both EISO and IB significantly reduced all these parameters in the Psoriasis skin samples (Sharma, in press).

Phosphodiesterase 4 inhibition

Using lipopolysaccharide (LPS) to stimulate different cell lines (human dermal fibroblast, BEAS-2B, A549, and THP-1 cells), it was shown that EISO inhibited the ability of PDEs to hydrolyze cAMP, decreased the transcription and activation of PDEs (PDE4 and 7), and suppressed the kappa B (NF-kB) activation as well as subsequent pro-inflammatory cytokines/chemokine production. Whatever cell line considered, the PDE activity increased significantly after LPS exposure, while co-treatment with EISO at 0.002%, suppressed LPS-mediated PDE activity dose- and time-dependently, and to levels comparable or below to that observed in the control sample (Sharma 2018).

11b-HSD1 activation

The role of sandalwood oil in induction of 11b-HSD1 was demonstrated in cultured normal human epidermal keratinocytes (NHEK). The same team investigated the response to various stimuli in NHEK treated by sandalwood oil and found that the expression of IL-1 induced by chemicals such as 2,4,6-trinitrobenzenesulfonic acid, 2,4-dinitrofluorobenzene (DNFB) or 2,4,6-trinitro-1-chlorobenzene (TNCB) was suppressed by sandalwood oil , confirming that sandalwood oil can suppress skin inflammation via 11b-HSD1 activation (Itoi-Ochi 2016).

2. <u>Clinical studies in inflammatory skin diseases</u>

ATOPIC DERMATITIS

A single-center, open-label, proof-of-concept Phase 2 trial investigated the safety and efficacy of a regimen of three topical EISO formulations containing 0.1% colloidal oatmeal for the treatment of AD/eczema. Of the 25 pediatric patients with mild, moderate, or severe AD who were enrolled 22 completed the treatment regimen, which combined bubble bath gel, EISO cream, and daily cleanser for 60 days. Outcome measures included changes in AD severity, as assessed by using the Eczema Area and Severity Index (EASI), with a 25% reduction in scores being the primary endpoint. The authors report that AD improved in all but 1 patient (who had an allergic reaction to carpet cleaning solution before the final visit). 87.5% of patients met the primary endpoint of the study (a 25% reduction in their EASI score) and there were no adverse events or safety issues with the treatment. The average reduction in overall scores was 67.8% (average of 6.2 EASI score reduction per patient), with 75% of patients achieving a >50% reduction in their score. 18.8% experienced a complete remission of their symptoms (a 100% reduction in EASI score) (Sharma 2018).

A randomized vehicle-controlled study assessed the safety and efficacy of EISO at 5 and 10% in 71 subjects with mild to moderate atopic dermatitis (BSA between 2 and 15%). Products were to be

applied twice daily for 4 weeks. At the end of treatment 36% of patients were cleared or almost cleared in the 10% group when they were 30% in the 5% group and 26% in the vehicle group.The change from baseline in EASI score in the ITT population and end of treatment was 63%, 43% and 19% for the 10%, 5% and vehicle respectively, confirming the results of the primary endpoint (unpublished Santalis data).

PSORIASIS

A single-center, open-label safety, tolerability and efficacy trial of a formulation of EISO at 10% for the treatment of mild to moderate plaque psoriasis was conducted in 12 adult subjects. The study medication was applied twice a day for up to 28 days. The average IGA score of the cohort was significantly reduced by 1 week on study and continued to improve at 2 and 4 weeks on study. Overall, 64% of the subjects demonstrated an at least 1-point reduction in their IGA score during the 28-day treatment period.

ACNE

A topical blend of salicylic acid and EISO was used in an open-label study in adolescents and adults with mild to moderate facial acne. The investigational regimen consisted of a foaming cleanser, an acne serum, a spot treatment, and a mask. Patients applied the treatment regimen as directed for 8 weeks. Results showed that 89.4% of subjects (42/47) met the primary end point determined by a global efficacy score of improved (66%), much improved (19%), or very much improved (4%). The treatment regimen was well tolerated by patients (Moy 2012).

RADIATION DERMATITIS

A clinical study assessed the effectiveness of sandal wood oil-containing cream in the prevention of radiation dermatitis in patients undergoing radiotherapy for head and neck cancer. A total of 50 patients with cancer requiring 60Gy of curative radiotherapy were enrolled and randomly divided into two groups of 25 patients (a group testing a classic oil preparation and a group testing the sandalwood oil preparation). Application of the cream was initiated on Day 1 and continued every day until 2 weeks after the end of treatment. A significant reduction in severity grades of dermatitis were seen in the cohort applying the sandalwood oil at all time points, including 2 weeks post radiotherapy. The occurrence of Grade 3 dermatitis was also significantly lower in this cohort, confirming the preventative and curative effect of sandalwood in this indication (Palatty 2014).

A second study of similar design was conducted with the same products in women undergoing curative radiotherapy for their breast cancer (50 Gy in 2 Gy fractions daily for 5 weeks). The endpoints were to ascertain the delay in the appearance and the degree of severity of dermatitis throughout the study period in accordance to the Therapy Oncology Group (RTOG) score. The results confirmed that the sandalwood-based product significantly delayed and mitigated the radiodermatitis (Rao 2017).

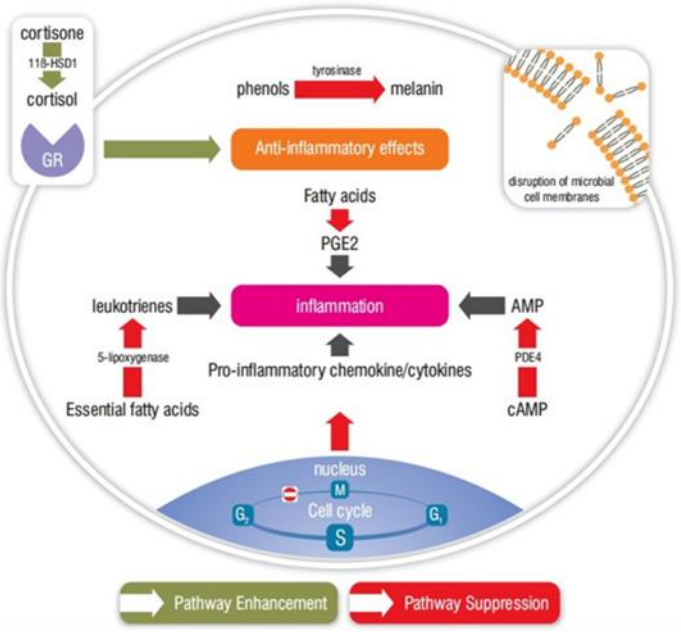
CHEMO AND RADIATION INDUCED MUCOSITIS http://dx.doi.org/10.14312/2397-8511.2020-1

A clinical study evaluated the efficacy, safety and tolerability of East Indian Sandalwood Oil (EISO) for the prevention and treatment of oral mucositis induced by radiation therapy with or without

concurrent chemotherapy. This was an open label exploratory study in adult patients with head and neck cancer planned to undergo high dose radiation therapy (i.e., ≥ 60 Gy) with or without concurrent chemotherapy or biologic targeted therapy. EISO was to be used as a mouth wash three times daily, 15 minutes after meals. The primary efficacy was to be assessed by the numerical rating pain scale (NRPS) and RTOG mucositis grade at Visit 7 (Day 36). Between October 2015 and April 2017, 13 subjects (11 males and 2 females, 42 to 78 years old) were included and 8 completed the study. In this population, grade 3 and 4 Oral Mucositis is reported to occur in about 70% of patients and severe pain (score ≥ 8) is reported by approximately 60% of patients. In this study these numbers were reduced to 25% and 37% respectively: only two patients had a mucositis score (RTOG score) of 3 or 4 and severe pain was reported by 3 out of 8 patients. In addition, mucosal infection was declared in only 2 subjects (25%) when the expected rate is closer to 70%. This protective effect could be explained by the antibacterial effect of Sandalwood oil in addition to its anti-inflammatory properties. (Ha CS,2020)

Conclusion

EISO demonstrated consistent and clinically relevant anti-inflammatory properties in in-vitro models as well as in clinical studies. Its polypharmacological profile is well investigated and targets 4 major pathways for skin biology: 5-Lipoxygenase, Cyclooxygenase-2, Phosphodiesterases and 11β-Hydroxysteroid dehydrogenase type 1. This very broad activity (amplified by the additional anti-oxydant activity) is unique and allows a positioning in many skin conditions and diseases as exemplified by the positive clinical studies summarized above.



Positioning and claims

Depending on the product and its regulatory frame, one can consider different options:

- Cosmetic products: claims could include skin homeostasis (or health) restoration or protection and anti-inflammatory effects could translate in soothing or management of redness, dullness or blemished skin. Products could also be presented as “suitable” for given diseases (example “appropriate for/suitable to skins with atopic tendency/atopic skin”)
- Cosmetic for skin diseases: breakout control, treatment of acne blemishes, help clear acne spots, calming, anti-irritating
- OTC products: EISO could be used as secondary ingredient in products that are already monographed (Example, EISO in a salicilyc acid formulation claiming efficacy in acne or EISO in an oatmeal product for eczema).

C. Anti-microbial properties of EISO

Rationale

The skin is a complex and dynamic ecosystem that is inhabited by bacteria, fungi and viruses. These microbes—collectively referred to as the skin microbiota—are fundamental to skin physiology and immunity. Interactions between skin microbes and the host can fall anywhere along the continuum between mutualism and pathogenicity. Microbes metabolize host proteins and lipids and produce bioactive molecules, such as free fatty acids, AMPs, phenol-soluble modulins (PSMs), cell wall components, and antibiotics. These products act on other microbes to inhibit pathogen invasion, on the host epithelium to stimulate keratinocyte-derived immune mediators such as complement and IL-1, and on immune cells in the epidermis and dermis.

Besides true skin infections, the composition of the skin microbiome can also shift markedly during inflammation. It is however not yet understood how pathogens and skin inflammation contribute to a vicious cycle, how homeostasis is re-established, or how pathogens interact with the existing commensal population. The clinical experience has however proven that microbes are good targets in the treatment of inflammatory skin diseases.

Indications of interest

INFECTIOUS SKIN DISEASES

Dermatophyte infections

Dermatophytosis is a superficial fungal infection with an outstanding morbidity rate in humans, affecting approximately 20% of the world population. More than 30 species of fungi, belonging to three main genera (*Epidermophyton*, *Microsporum* and *Trichophyton*), have been identified as causative agents, but *Trichophyton rubrum* is the most frequent species in humans. These fungi are characterized by invading the stratum corneum and other keratinized tissues like nail and hair, where they thrive by secreting enzymes and degrading keratin to obtain nutrients, also promoting tissue damage. Patients with acute superficial dermatophytosis generate cell-mediated immune responses against the causative agent, which, combined with medical treatment, are associated to the resolution of the infection. However, a significant number of subjects are unable to develop this response and suffer from chronic or recurrent infections, thus needing preventative approaches.

Herpes infections

Herpes simplex virus (HSV) is differentiated into two antigenic types of type 1 (HSV-1) and type 2 (HSV-2). HSV infects and replicates in cells at the site of entry (mucocutaneous surface) and is then transported through retrograde axonal transport to cell bodies of neurons in sensory ganglion where the virus will persist for life-time. HSV-1 is a highly prevalent pathogen among children and adults, causing primary infections which present clinically as herpes labialis or as primary herpetic gingivostomatitis, and is able to establish a latent infection in the nervous system that can be reactivated quite frequently. Genital herpes (HSV2) is one of the most prevalent sexually transmitted disease worldwide and is the most common cause of genital ulcers. What makes HSV so difficult to

control is that most sexual and perinatal transmissions occur during unrecognized or asymptomatic shedding.

Warts and molluscum contagiosum

Viral warts are common skin conditions seen in both children and adults and caused by Human papilloma virus (HPV), a DNA virus. It is a highly prevalent skin condition, especially in children (33%) and organ transplant recipients (45%). Thirty percent of warts may clear spontaneously, but those that do not are often cosmetically unappealing, painful, and irritating to the patient. There are several treatments for plantar verrucae; however, none of them are specific to HPV. They include nonsurgical and surgical methods, such as the use of keratolytic agents (salicylic acid), cryotherapy (liquid nitrogen) or laser (pulse dye and CO2).

Molluscum contagiosum (MC) is a self-limited infectious dermatosis, frequent in pediatric population, sexually active adults, and immunocompromised individuals. It is caused by molluscum contagiosum virus (MCV) which is a virus of the Poxviridae family. MCV is transmitted mainly by direct contact with infected skin, which can be sexual, non-sexual, or autoinoculation. The duration of the lesions is variable, but in most cases, they are self-limited in a period of 6-9 months. However, some cases may persist for more than 3 or 4 years.

INFLAMMATORY DISEASES

Atopic dermatitis

The pathology of AD includes three critical factors: impaired skin barrier function, microbial dysbiosis, and cutaneous immune abnormality predisposed to T helper 2 (Th2) immunity, which may aggravate one another. Many studies have found alterations in the composition of the microbiome in patients with AD compared with that of healthy individuals, and microbiota differs between lesional skin and non-lesional skin. A meta-analysis of 95 observational studies showed that the pooled S. aureus colonized 70% of lesional skin of AD patients, 39% of nonlesional skin of AD patients and only 3% of nonatopic patients (Totte 2016). Dysbiosis in microbiota with particular attention to S aureus has been universally considered an important factor in the pathogenesis of AD, and how to achieve and maintain a balance between skin microbiota and the host has become a hot research topic in the field of AD treatment with the expectation of obtaining long-term remission.

Presume human data?

Acne

Increased sebum production, inflammatory mediators of the skin, and follicular keratinization of the pilosebaceous ducts are believed to contribute to acne development. Colonization by Cutibacterium acnes (C. acnes; formerly called Propionibacterium acnes) is also recognized in acne patients, but its role is unclear because it is ubiquitous in the sebaceous areas of healthy skin from puberty onward. Several mechanisms have been proposed by which C. acnes aggravates acne, including augmentation of lipogenesis, comedone formation, and host inflammation. Based international treatment guidelines and expert consensus, antibiotics (macrolides, clindamycin, and tetracyclines) are recommended as the first-line therapy in acute infammatory acne. However the growing prevalence of antibiotic resistance in C. acnes and other skin commensal bacteria is becoming increasingly alarming.

Wounds

Skin damage can be caused by a variety of different reasons such as trauma (including cuts, abrasions, chemical burns, fire burns, cold, heat, radiation, surgery), or as a consequence of underlying illnesses such as diabetes. The most effective wound management strategy is to prevent infections, promote healing, and prevent excess scarring. Chronically infected wounds, such as venous or arterial ulcers, diabetic foot ulcers, pressure sores, and nonhealing surgical wounds delay wound healing, have a significant impact on the patients' quality of life, represent a significant cause of morbidity and mortality, and result in enormous healthcare expenditures. Wound infections are most often caused by biofilm-forming bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Escherichia coli* or *Klebsiella pneumoniae* (Fijan 2019).

Others

The generation of malodour on various sites of the human body is caused by the microbial biotransformation of odourless natural secretions into volatile odorous molecules. Corynebacterium has since been implicated as the most likely contributor to axillary odor through the biotransformation of natural human secretions to produce medium chain volatile fatty acids and thioalcohols (James 2012).

Dandruff, or seborrheic dermatitis, is one of the most common skin conditions afflicting humans. This chronic hyper-proliferative inflammatory condition typically results a red and flaky appearance of the scalp and/or sebaceous areas of the face and sometimes chest. While the etiology of this condition remains poorly understood, it has long been associated with the Malassezia yeasts (Wikramanayake 2019).

Demodex folliculorum are found in rosacea patients with the papulopustular subtype at a population density of more than 5x that of a healthy patient. In rosacea, the Demodex mite could act as a vector for the bacteria, Bacillus oleronius. It has been hypothesized that the mites cause the distention of the follicles, allowing for the bacteria to be introduced to the innate immune system through the pilosebaceous unit and a subsequent mononuclear cell proliferation and induction of neutrophils follows. Statistically significant serum immunoreactivity against proteins isolated from B oleronius was demonstrated in both papulo-pustular and erythemato-telangiectatic rosacea subtypes (Holmes 2013).

Data available with Sandalwood oil

SAO has shown activity against a range of bacteria, yeasts and fungi associated with skin disease as shown in table below:

Gram positive bacteria	
Micrococcus flavus	*Staphylococcus aureus*
Micrococcus glutamicus	*Staphylococcus aureus (MRSA)*
Propionibacterium acnes	*Staphylococcus epidermidis*
Sarcina lutea	*Streptococcus equisimilis*
Staphylococcus albus	*Streptococcus pyogenes*
Gram negative bacteria	
Acinetobacter baumannii	*Pseudomonas aeruginosa*
Acinetobacter calcoaceticus	*Pseudomonas florescens*
Klebsiella aerogenes	*Pseudomonas putida*
Klebsiella pneumoniae	
Yeasts & fungi	
Candida albicans	*Microsporum gypseum*
Candida krusei	*Trichophyton asteroides*
Epidemophyton fluccosum	*Trichophyton interdigitale*
Epidemophyton inguinale	*Trichophyton mentagrophytes*
Microsporum canis	*Trichophyton purpureum*

In a study evaluating the in vitro antimicrobial properties of 59 commercial essential oils against a broad spectrum of pathogens of dermatological relevance (S. epidermidis, S. aureus, MRSA, E. coli, P. aeruginosa, Propionibacterium acnes, Candida albicans, Trichophyton mentagrophytes, Microsporum canis, Brevibacterium agri, B. epidermidis, B. linens), Sandalwood oil revealed to be effective on all strains tested, with MIC < 2 mg/ml (Orchard 2017).

BACTERIA

Staphylococcus aureus

The incidence of hospital-acquired infections with antibiotic-resistant bacteria has increased remarkably. Notable amongst these infections is methicillin-resistant Staphylococcus aureus (MRSA) which has important epidemiological, financial and logistical implications. A study evaluated antibacterial and antimycotic efficacy of different essential oils on frequently isolated and hospital-acquired bacterial strains including MRSA by means of the agar diffusion test. Sandalwood oil showed good efficacy (similar to ethanol 70%) against the S aureus, S epidermidis and Streptococcus strains, but not against the Candida strains.

Pseudomonas aeruginosa

The antibacterial activity of Santalum album wood extract was recorded against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Proteus vulgaris. A cold water extract showed a zone of inhibition (15 mm) against P. vulgaris followed by B. subtilis (14 mm), E. coli (10 mm), and P. aeruginosa (9 mm) (Khan 2013).

Cutibacterium acnes (formerly Propionibacterium acnes)

The antibacterial activity of 70 selected essential oils was assessed using the minimum inhibitory concentration (MIC) assay against Staphylococcus epidermidis (ATCC 2223) and Propionibacterium acnes (ATCC 11827). The MIC for C acnes with Santalum album oil was 0.69 mg/ml.

Sharma tested the efficacy of East Indian Sandalwood oil (EISO), benzoyl peroxide (BPO) and BPO plus EISO to suppress production of pro-inflammatory cytokine and chemokines by P. acnes in a three-dimensional normal reconstituted skin model prepared from normal human epidermal keratinocytes and fibroblasts. The standard ATCC (American Type Culture Collection) strain of P. acnes and five clinical isolates of P. acnes were used to colonize and stimulate the skin samples in the absence or presence of EISO (45 and 90 µM), BPO (2.5 and 5%) or Erythromycin (90 µM) for 2, 3 and 8 days. Cell-free supernatants were analyzed by ELISA assays for cytokine IL-6 and chemokine IL-8 (incubation with P. acnes was shown to substantially increase both cytokines). IL-6 and IL-8 levels were not affected by Erythromycin or BPO treatment at the indicated doses, however they were dose-dependently suppressed by 45 µM and 90 µM EISO (Sharma, in press).

VIRUSES

HSV1 and HSV2

Sandalwood oil was tested for in vitro antiviral activity against Herpes simplex viruses -1 and -2. A virus sample containing 10^6 PFU/ml was incubated at 37°C for 1 h with a media containing different non-cytotoxic concentrations of sandalwood oil (from 7.5 to 60µ/ml) or media alone (controls). Thereafter, samples were diluted to determine residual infectivity by the plaque formation assay. It was found that the replication of these viruses was inhibited in the presence of the oil. This effect was dose-dependent and more pronounced against HSV-1 (Benencia 1999).

Another experiment tested the sensitivity of acyclovir-sensitive HSV-1 strain as well as acyclovir-resistant patient isolates to sandalwood oil. It confirmed that Sandalwood oil was viricidal in both strains (EC50 at 0.0002 %) with a mechanism that was different from acyclovir. The oil seemed more efficient on cell-free virus but had limited effects on virus replication in cells and on the cell-to-cell spread of the virus, suggesting a direct effect on the HSV envelope (Schnitzler 2007). Similar conclusions were drawn from another experiment assessing sensitivity of HSV2 strains (Koch 2008)

HPV

The effectiveness of sandalwood oil as a topical treatment for common warts was investigated in a pilot open trial. Ten subjects were enrolled and asked to apply a drop of sandalwood oil onto the wart area twice a day for 12 weeks. At the end of the study, 8 of 10 (80%) had complete resolution of all treated warts. The remaining 2 subjects had improvement rated as moderate (25% to >90%). There were no complaints of skin irritation, erythema, itching, pain or discomfort, or other adverse events reported (Haque 2017).

Another study was conducted by Santalis in 183 patients > 16 years old enrolled in a classical Phase 2 dose ranging study with 4 arms, 10%, 20%, 30% and placebo. Products were applied twice daily for 12 weeks on 1 to 10 warts in an area of less than 25 cm2. Results were not totally conclusive with the 10% concentration being the most effective concentration for clearing rate and prevention of new lesions.

Molluscum contagiosum

In another open label pilot study conducted by the same team, Sandalwood oil was tested for its efficacy in 10 subjects with molluscum contagiosum applying the product twice daily for 12 weeks. Nine of ten subjects (90%) experienced complete resolution (Haque 2017)

YEASTS AND FUNGI

In a study investigating the biologically active natural products from heartwood of Santalum album, alfa-santalol was shown to have significant antifungal effect against Trichophyton rubrum with a mechanism of action (mitosis inhibition) and an efficacy similar to that of Griseofulvine (Kim 2012).

Conclusion

Sandalwood oil carries broad-spectrum antimicrobial properties by mechanisms that are not fully understood but might be related to the disruption of membrane or structure integrity, a phenomenon that has been demonstrated for other essential oils. Its efficacy on resistant strains, especially MRSA is to be noticed as well as the significant effect on Dermatophytes. Its very good safety profile reported in clinical trials supports the use in many skin diseases particularly in chronic conditions and preventative approaches. The activity of EISO should be further studied for strains such as Corynebacteria, Malassezia species and Bacillus oleronius to expand the field of applications.

Positioning and Claims

Skin disinfection

Treatment of tinea pedis and Prevention of reinfestation

Prevention of herpes virus dissemination

Treatment of benign viral infections such as warts and molluscum contagiosum, prevention of dissemination

Staph decolonization in folliculitis or chronic staphylococcal porterage

Prevention of flare in Atopic dermatitis

Wound disinfection

Wound healing

Treatment of acne

D. Anti-proliferative properties of EISO

Rationale

"Proliferative skin disease" is a very general terminology defined to include all skin diseases characterized by an abnormal proliferation of epidermal keratinocytes. Such diseases include skin conditions as different as psoriasis, cancer, eczema, or ichthyosis. In this section we will only address pathologies related to non-melanoma skin cancer, including the precancerous stages such as Actinic keratosis.

Worldwide, Non-Melanoma-Skin-Cancer (NMSC) is the most frequent type of skin cancer in Caucasian populations, registering an increased incidence in the last 40 years. This rapid increase is based on UV radiation exposure accounting for 90% of NMSC cases. Although the incidence of other malignancies has stabilized or even declined, the incidence of NMSC has increased constantly with a younger patient age at diagnosis, representing a main public health concern. Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most frequent NMSC, registering the highest world incidence in Auckland where rates were 425 for SCC and 1,177 for BCC per 100,000 individuals per year (Pondicherry 2018).

Among the main environment aggressors, the UV radiation (UVA and UVB) from the sun induces several biological effects, high DNA damage and reactive oxygen species (ROS) generation. In experimental models, the initial step in UVB-induced carcinogenesis involved induction of DNA damage (e.g. cyclobutane pyrimidine dimer and pyrimidine pyrimidone photoproduct formation), which triggers a rapid increase in p53, enhancing Cip1/p21 synthesis and shutting off cell replication and DNA synthesis, allowing extended time for either DNA repair or apoptosis induction in the cells carrying UVB-damaged DNA. Whereas this defense mechanism efficiently removes UVB-damaged cells from the skin epidermis, sometimes damaged cells escape both repair and apoptotic death finally leading to transformed cells known as UVB-initiated epidermal keratinocytes. These cells follow multiple additional hits by repeated UVB exposure finally leading to benign followed by malignant skin tumors (Dwivedi 2006).

In skin there is a clear connection between tissue destruction, inflammation and tumor onset. Regardless of the origin of the environmental factors, the maintenance of an inflammatory microenvironment is a clear condition that favors tumorigenesis. Inflammation sustains the proliferation and survival of malignant transformed cells, can promote angiogenesis and metastatic processes, can negatively regulate the antitumoral adaptive and innate immune responses and may alter the efficacy of therapeutic agents.

Indications of interest

Precancerous lesions

Data from 11 clinical studies support this experimental report by showing a 10% risk reduction of BCC for patients using NSAIDs. This analysis highlights that in humans COX inhibition via NSAIDS can be used in high-risk populations to reduce BCC initiation (Muranushi 2016).

Actinic keratoses

Actinic keratoses (AKs) are diagnosed at > 10% of dermatology visits, making them one of the most common diagnoses seen in the outpatient setting. In 2004, in the U.S.A., AK prevalence was estimated at almost 40 million, with annual costs > $1 billion US$ (Bickers 2006). AKs are problematic because they can develop into SCC and certain authors consider that it is already a SCC at an *in-situ* stage.

Clinically, AKs are described as asymptomatic erythematous or flesh colored, scaly papules or plaques with a gritty, sandpaperlike texture that is often more easily felt than seen. AKs range in size from a few millimetres to > 2 cm in diameter, often coalesce, and are usually found in sun-exposed areas such as the face, dorsum of the hands, shoulders and balding scalp.

Treatment for AK primarily consists of lesion-directed destruction or field treatment with topical medications which aim to prevent progression to SCC. Cryotherapy, mainly for reimbursement and practicality reasons, remains the standard of treatment in Western countries, although it is painful and does not address the field of cancerization question.

Data available with Sandalwood oil

In vitro Research data

In vitro studies have investigated the mechanisms involved in the tumorigenesis prevention effects of Santalols:

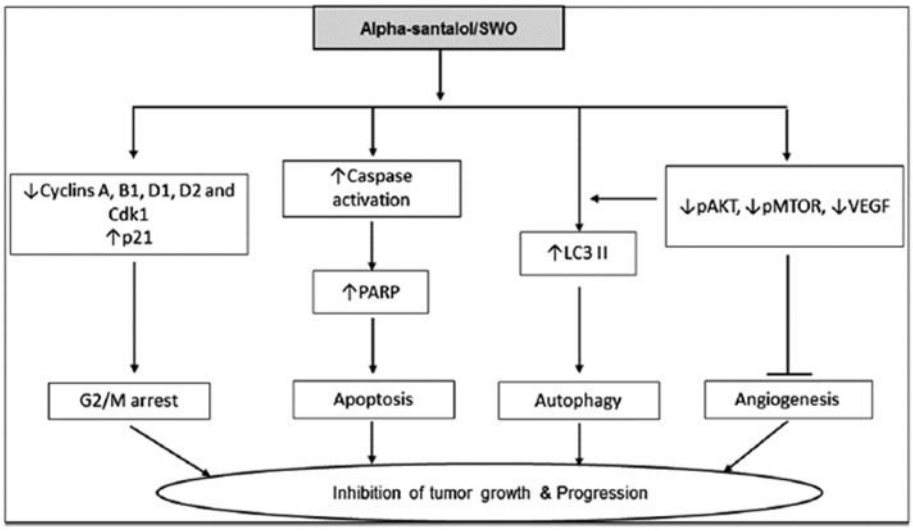
- the most prominent mechanisms by which alpha-santalol has its cytotoxic effects against cancer cells are through induction of cell-cycle arrest and apoptosis. α-santalol induced apoptosis cell death in breast cancer cells (regardless of their estrogen receptor or p53 status) while producing fewer toxic effects on a normal breast epithelial cell line (Santha 2013). This effect was mediated through cell cycle arrest at G2/M phase and induction of apoptosis through both extrinsic and intrinsic pathways and activation of caspase-8 and caspase-9 (NB: Apoptosis is controlled by the extrinsic death receptor pathway and the intrinsic mitochondrial pathway. Notably, both pathways converge at caspase-3, leading to activation of other proteases).

- α-santalol also inhibited human cancer cell growth (epidermoid carcinoma A431 and human melanoma UACC-62 cells) *in vitro* through blockade of mitosis. Two mechanisms were identified: up-regulation of cyclin B and down-regulation of both cyclin A and cyclin B complexes resulting in microtubule depolymerization (Zhang 2010).

Similarly, EISO-treatment of HaCaT keratinocytes resulted in a blockade of cell cycle progression as well as a concentration-dependent inhibition of UV-induced AP-1 activity, two major cellular effects known to drive skin carcinogenesis. Induction of autophagy was suggested. (Dickinson 2014).

Mechanisms underlying inhibition of tumour growth and progression by Sandalwood oil

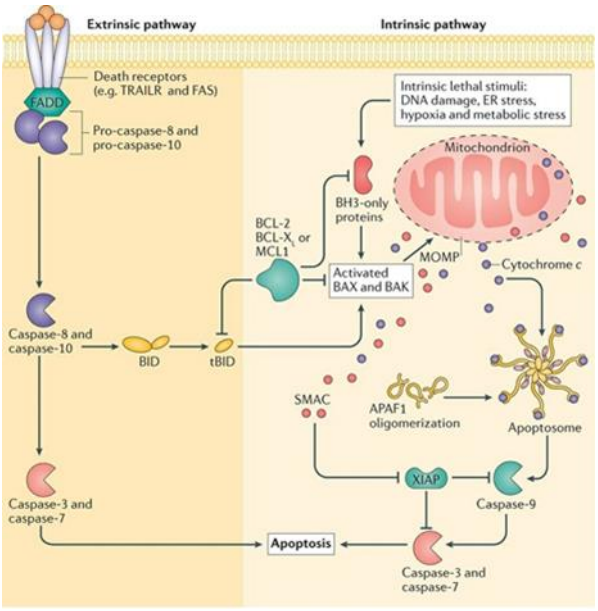
From Bommareddy 2019



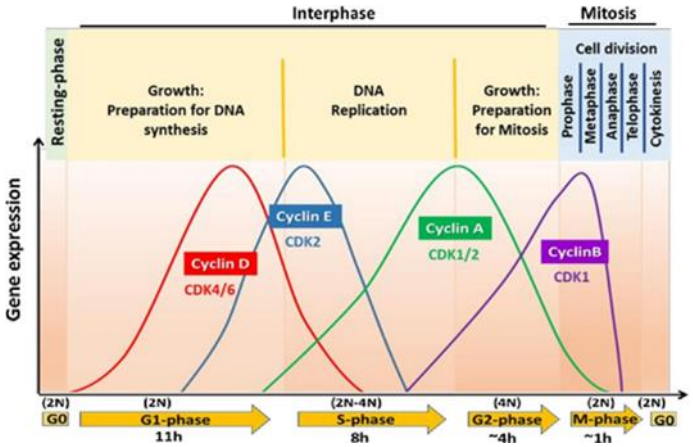
Mechanisms involve: (i) Growth arrest at G2/M phase of cell cycle due to down regulation of cyclins, cdk1 and induction of p21 (Zhang et al. 2010; Santha and Dwivedi 2013; Santha et al. 2013; Lee et al. 2015) (ii) Apoptosis induction leading to caspase activation and cell death (Bommareddy et al. 2012; Santha et al. 2013) (iii) induction of autophagy (Dickinson et al. 2014) (iv) Inhibition of angiogenesis due to suppression of p-AKT and VEGF signaling (Saraswati et al. 2013).

The intrinsic and extrinsic pathways of apoptosis

https://biologydictionary.net/apoptosis/



Role of Cyclins in the Cell cycle https://www.semanticscholar.org/



Eukaryotic cell cycle. The cell cycle comprises two major phases, interphase and mitosis. Interphase, the longer phase of the cell cycle, is divided into three sub-phases: G1 phase, S phase and G2 phase. The G1 phase can last up to 11 h, the S phase, or the DNA synthesis phase lasts up to 8 h, and the G2 phase lasts up to 4 h. The mitotic phase (M) is the shortest phase in the cell cycle. It is further divided into prophase, metaphase, anaphase, and telophase.

Cyclin D triggers cell to move from G0 to G1 and S phase. Cyclin E prepares the cell for DNA replication. Cyclin A activates DNA replication. Cyclin B promotes the assembly of the mitotic spindle to prepare for mitosis.

Conclusion

The chemopreventive effect of Sandalwood oil and its main component, alpha santalol, is by far the most studied property of this product. Many *in vitro* and *in-vivo* studies have confirmed the ability of Sandalwood oil or Santalols to prevent skin cancers.

Positioning and Claims

Potential positionings

Prevention of skin cancers in subjects with photoaging

Prevention of skin cancers in immunocompromised patients (organ transplant recipients)

Treatment and prevention of actinic keratoses

Treatment of field of cancerization in subjects with multiple actinic keratoses

Potential claims

Helps to prevent skin cancers in addition to photoprotective measures

Prevention of various forms of severe cutaneous diseases in patients at risk

Reduce the risk of actinic keratosis lesions

Prevents and repairs the actinic damage

Reduces and improves the sublinical cutaneous cancerization field associated with actinic keratoses (AK) and non-melanoma skin cancer (NMSC).

E. Anti-pigmenting properties of EISO

Rationale

Disorders of hyperpigmentation such as melasma and post-inflammatory hyperpigmentation are common reasons for visits to dermatology practices: it is estimated that approximately 15% of the population in the world invests for skin whitening and that the worldwide market for skin whitening agents will reach nearly U.S. $23 billion by 2020 (https://cosmetics.specialchem.com/news/industry-news/skin-lightening-products-market-to-reach-usd23-bn-by-2020-global-industry-analysts).

Inhibition or reduction of melanin synthesis by overactive melanocytes is the common mechanism for most of the whitening agents available in the market. Complex enzymatic and biochemical-catalyzed reactions are involved in melanin synthesis. Enzymatically, tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1), and tyrosinase-related protein-2 (TRP-2, also called dopachrome tautomerase) are three key regulators of the melanogenesis.

Oxidative stress is a contributing variable that appears to be a significant player in melanogenesis. It is well -established that UVA irradiation, especially UVA1 (340 -400 nm), induces pigmentation and oxidative stress to a greater extent than UVB (290 -320 nm) irradiation. However, approximately 50% of all free radicals generated in the whole solar spectrum can already be induced by radiation in the Visible and Near Infrared regions. It is important to understand that chemical sunscreens do not protect from those wavelengths and that mineral filters are only minimally effective.

Indication of interest

Skin Brightening

Skin brightening (SB), involves the use of topical products that contain corticosteroids, hydroquinone, mercury, and/or a variety of other agents to attain a lighter skin color. The motivation for SW may be to treat a pigmentary disorder, such as melasma, but is often simply for cosmetic enhancement. The cosmetic use of SW products is common in Africa, Asia, and many other parts of the world. A recent meta-analysis reported a global pooled lifetime prevalence of use of skin bleaching agents of 27.7%. The authors warned that these results represent a serious global public health problem (Sagoe 2019).

Numerous cutaneous adverse effects (e.g., atrophy, striae, telangiectasias, acne vulgaris, allergic and irritant contact dermatitis, hirsutism, hypertrichosis, perioral dermatitis, steroid rosacea, dyschromias, ochronosis, and infections) and even systemic complications (e.g., Cushing's syndrome, diabetes, hypertension, and ocular changes [cataract and glaucoma]) have been associated with the abuse of skin lightening agents. There is thus a need for new products with excellent safety profiles compatible with long term use.

Melasma

Melasma is a human melanogenesis dysfunction that results in localized, chronic acquired hypermelanosis of the skin. It occurs symmetrically on sunexposed areas of the body, and affects especially women. It is known that melasma occurs in all ethnic and population groups. However, epidemiological studies have reported higher prevalence among more pigmented phenotypes, such as East Asians (Japanese, Korean and Chinese), Indian, Pakistani, Middle Eastern and Mediterranean-African. Melasma distresses patients since it affects the face, being easily visible and constantly present in everyday life. In this context, it has a negative impact on the quality of life of patients, affecting their psychological and emotional well-being, which often motivates them to search for a dermatologist.

The etiology of melasma is multifactorial. UV light has been shown to trigger and exacerbate the condition through the induction of reactive oxygen species (ROS). Patients with melasma have been found to have higher markers of oxidative stress when compared to healthy volunteers. More recently, the role of visible light in inducing pigmentation has been appreciated.

Post-inflammatory hyperpigmentation

Postinflammatory hyperpigmentation (PIH) is an acquired hypermelanosis occurring after cutaneous inflammation or injury that can arise in all skin types, but more frequently affects skin-of-color patients. Many types of inflammatory dermatoses or cutaneous injuries can cause pigmentary changes; however, there are some diseases that show a proclivity to develop PIH such as acne vulgaris, atopic dermatitis, and impetigo. A study in 2002 evaluating acne in skin of color found that 65.3 percent of African American, 52.7 percent of Hispanic, and 47.4 percent of Asian patients developed acne-induced PIH (Taylor 2002).

Data available with Sandalwood oil

In an anti-tyrosinase assay using mushroom tyrosinase, a popular target enzyme for screening and characterizing potential tyrosinase inhibitors, sandalwood oil showed activity with IC50 at 174 µg/mL. In the same assay, kojic acid (IC50, 5.4 µg/mL) and tropolone (IC50, 0.7 µg/mL) showed stronger inhibition activities, whereas arbutin led to similar levels of inhibition (IC50, 149 µg/mL) (Misra 2012).

Sandalwood oil is used as an ingredient in numerous skin fairness enhancing cosmetics and described in Ayurvedic medicine as Chandana. In combination with other plant extracts and under the name of Varnya *Gana Lepa* (10 drugs paste), it showed significant efficacy (MASI reduction) in 40 subjects with melasma in only 15 days (Pallavi 2015).

Conclusion

The combination of direct tyrosinase inhibition and anti-oxidant activity support the use of Sandalwood oil for the treatment and prevention of pigmentary disorders.

Positioning and Claims

Potential positionings

Safe bleaching agent (probably in combination with other ingredients)

Treatment of melasma and prevention of relapses (ideally in combination with a high UVA + VL filter)

Treatment and prevention of hyperpigmentation in acne or following procedures (lasers, peelings)

Potential claims

Depigmenting and unifying agent

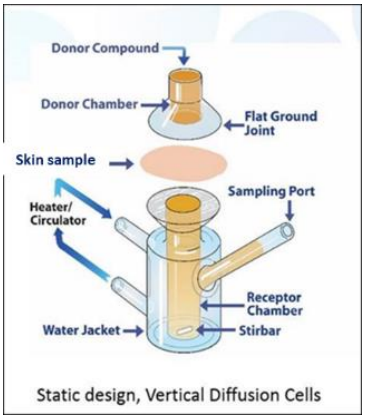
Reduce the size and intensity of brown spots

Combats the over-production and poor distribution of pigment in the skin

F. FORMULATION WORK WITH EISO

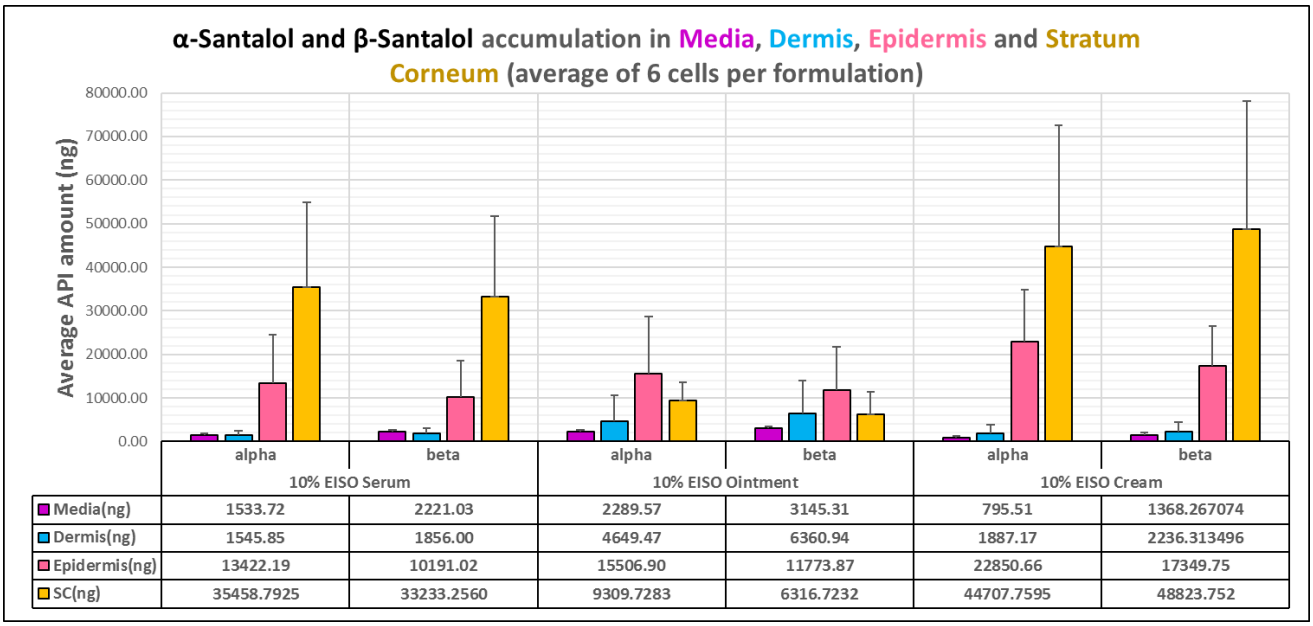
The efficiency of a topical treatment depends on the ability of the formulation components to be released from their vehicle and to penetrate through the first layers of the skin at effective concentrations. The skin, and particularly its outermost layer, stratum corneum (a part of the epidermis made up of 10 to 30 thin layers of the dead cells), is an effective mechanical barrier to the permeation of external molecules. Before evaluating the efficacy of a new product in a clinical trial, it is therefore strongly recommended to confirm the formulation performance in in-vitro or, better, ex-vivo models of skin permeation. The in vitro vertical diffusion cell system with human skin and finite dose model is a valuable tool for the study of percutaneous absorption and evaluation of the pharmacokinetics of topically applied products.

Quintis evaluated the penetration of alpha and beta santalol, the two identified actives in Sandalwood oil, from three different topical formulations containing 10% Sandalwood Album oil: a serum, an ointment, and a cream. The model used cryopreserved, dermatomed, ex vivo human cadaver torso skin mounted on vertical diffusion cells. Each formulation was dosed once with 15 µL/cm2 of the product applied to the skin using a positive displacement pipette. After 24 hours, the skin was gently washed, tape-stripped to remove un-absorbed formulation and heat split to epidermis and dermis layers. The santalols levels into and through the skin (6 replicates per formulation) were then measured using LC-MS/MS. (Bindu 2017)



Static design, Vertical Diffusion Cells

Evaluation of the data concludes that α- and ß-santalol effectively permeate into and through human skin from all three formulations. The amount of total santalol reaching various skin depths was dependent on formulation with the ointment being the most performant vehicle. For all three formulations, only a small amount of santalols passed through the skin into the receiving media (less than 0.5 % on average).



	10% EISO Serum		10% EISO Ointment		10% EISO Cream	
	alpha	beta	alpha	beta	alpha	beta
Media(ng)	1533.72	2221.03	2289.57	3145.31	795.51	1368.267074
Dermis(ng)	1545.85	1856.00	4649.47	6360.94	1887.17	2236.313496
Epidermis(ng)	13422.19	10191.02	15506.90	11773.87	22850.66	17349.75
SC(ng)	35458.7925	33233.2560	9309.7283	6316.7232	44707.7595	48823.752

Conclusions

The data of this experiment suggest that both α- and ß-santalol effectively permeate human skin, and that the observed concentrations are considered sufficient to elicit measurable pharmacological response. The results further establish that different topical formulations can deliver santalol to different skin depths to optimize efficacy for specific pharmacological targets. The low levels reported in the receiving media can predict very low systemic exposure and no general safety concerns in subjects treated with these formulations.

CONCLUSIONS

Sandalwood oil has been used since antiquity as a medicine, making it the probably oldest treatment in Humanity. Its multiple properties have made it a popular traditional remedy utilized by cultures across the world for diverse skin problems and as such Sandalwood oil can be found in numerous therapeutic applications in Chinese traditional medicine or Ayurveda.

A large body of literature from in vitro and in vivo studies report the chemopreventive effects of santalols or SO and their non-toxic side effects against normal cells. The most prominent mechanisms by which they have their cytotoxic effects against cancer cells are through induction of cell-cycle arrest and apoptosis. Animal studies demonstrate that topical application of α-santalol significantly reduces tumor incidence and tumor multiplicity in both chemical induced and UVB-induced skin cancer. These anticancer properties of santalols was further studied against various cancer models and include now targets beyond dermatology with initiatives to develop such compound as a drug.

Inflammation has a physiological important role in the skin and its final goal is tissue damage healing. When this process develops as chronic inflammation it triggers molecular and cellular networks that disturb the normal interactions between resident skin cells and immune cells and further alter tissue homeostasis. SO and santalols have shown their ability to interfere with important inflammatory processes and demonstrated usefulness in several clinical studies.

The skin is colonized by a large number of microorganisms, most of which are beneficial or harmless. However, disease states of skin can result from microbial invasion (infections) or from altered compositions leading to inflammatory skin diseases. The anti-microbial effect of EISO is well documented on most of the species involved in cutaneous pathologies and supports its use as a preventative or curative option.

The anti-oxidant and depigmenting properties are less supported by the evidence. However, the scientific rationale seems reasonable and further experiments should be conducted to better characterize these potential effects and their subsequent use in Dermatology.

Sandalwood oil appears easy to formulate in various semi-solid formulations and both α- and ß-santalol effectively permeate human skin with reported concentrations adequate to elicit measurable pharmacological responses.

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