Adaptogen Properties of Indian Sandalwood Oil in Skin Protection

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Abstract

Introduction: Indian sandalwood oil (ISO) has been found to act as a potent adaptogen for the skin, providing protection from modern day environmental stressors, the proposed mechanism of action is though quenching of reactive oxygen species (ROS). This finding presents very real opportunities for ISO in cosmetic preparations seeking to offer anti-aging or protective claims.

Indian sandalwood oil (ISO) is the essential oil from the aromatic heartwood of the *Santalum album* tree, which has been revered as a medicine, fragrance, cosmetic and a religious material for millennia. Sweet woody aroma and myriad of skin benefits of ISO are attributed to its major constituents, alpha and beta santalol. In cosmetics and perfumery, sandalwood is seen as a multifaceted ingredient giving aroma, fixative properties, bioactivities on skin and psychological benefits.

Modern lifestyle faces number of exposomes such as pollution, solar and digital blue light which trigger oxidation of skin tissue. Skin oxidation is a cascade of reactions resulting ageing, pigmentation, and inflammation.

Methodology: ISO was tested on keratinocytes stimulated by cigarette smoke, blue light at 412nm and 450nm. Generation of reactive oxygen species (ROS) was measured by oxygen radical fluorescent assay. Human skin explants treated with ISO were exposed to ozone and cigarette smoke to determine the oxidative stress. Investigations were further extended to an intra-individual comparative clinical study in which a simple formulation containing ISO was tested topically on the back of volunteers who were subjected to blue light and pollution stimulation. The protective effect of ISO was determined by levels of squalene mono-hydroperoxide assessed in sebum samples collected from treated and untreated areas.

Results: ISO was found to reduce the reactive oxygen species by 34% than the positive control α-tocopherol in human keratinocytes exposed to blue light and pollution. Clinical study has reported a significant reduction of squalene mono-hydroperoxide with topical application of ISO at 1 and 10% concentrations.

Discussions and Conclusion: ISO was found to act as an adaptogen in protecting the skin from oxidative stress and subsequent damage to skin. Moreover, ISO was found to be as effective and sometimes superior to the well-established topical antioxidant α-tocopherol. This effect was reported in vitro experiments and confirmed in a clinical study in healthy volunteers. ISO is traditionally used as a fragrance ingredient in cosmetic and personal care formulations: it is now to be regarded as a potent adaptogen with clear benefits on skin health.

Key words

Indian sandalwood oil; *Santalum album*; adaptogen; antioxidant; skin protection; blue light

Introduction

Human skin is constantly exposed to external stressors daily contributing to develop signs of premature aging. These external factors can be of chemical or physical in nature. Exposure to pollution and sun are considered the most damaging exposomes for the human skin, causing pigmentation, dullness, dryness, and overall reduction of appearance [1].

Blue light is the short wavelength portion of the visible light closer to the UV spectrum within the wavelength range of 400-480 nm. Visible light from sun emits blue light which is closer to the UV light at an average of 412 nm. Digital screens such as computer monitors, and mobile phone screens emits a less damaging longer wavelength averaged as 450nm. Prolonged exposure to blue light from sun and digital screens have identified to have detrimental effects on skin [2].

Pollution is a public health concern effecting more than half the world's population living in cities and industrial areas. The most common form of pollution is the air pollution which is primarily contributed by industrial and motor vehicle generated smoke. Smoke consists of particulate matter usually at a particle range of 2.5 micron and obnoxious gases such as carbon monoxide, sulphur dioxide and various volatile organic compounds [3].

Human skin reacts to blue light and pollution by producing reactive oxygen species (ROS) as defence mechanism, however, excessive exposome would produce surplus ROS would trigger a cascade of biochemical reactions in the skin. Therefore, oxidation of skin is considered as the starting point for skin aging, inflammation, and pigmentation. Inhibiting or limiting of oxidation can be desired initiative to protect the skin. Quantification of oxidation is considered as an important measurement of skin protection in both in-vitro and in-vivo studies. Reactive oxygen species can be directly measured in cellular cultures using a suitable dye and spectroscopical methods. More indirect measures are performed on human skin such as oxidation of squalene [4].

Adaptogen are compounds which help body to adopt to an environmental stress. Adaptogens acting on skin are primarily antioxidants which neutralises the oxidative stress to supress any further damage to the skin. Therefore, adaptogens are of high importance to cosmetic and personal care targeting skin protection [5].

There are several natural products which act on human skin my neutralising excessive ROS generated by exposome exposure. Commonly used antioxidants such as polyphenols, polyhydroxy acids and conjugated structures act by scavenging ROS by sacrificing a pair of electrons. The other group of antioxidants act by upregulating the skins own oxidation mechanism by facilitating synthesis of enzymes such as superoxide dismutase [6].

Indian sandalwood is one of the oldest cosmetic ingredients known to humankind. Skin benefits of sandalwood was first recorded in Ayurveda lexicon by Charaka in 200 BC, where sandalwood is considered to improve the skin radiation. Indian sandalwood has been used for various ailments as a medicine since antiquity in Ayurveda and traditional Chinese medicine. Traditional cosmetic uses of sandalwood comprised of face masks and treatments made from powder paste, these applications were traditionally used to reduce the appearance of acne and to protect and sooth the skin from harsh sunlight [7].

Indian sandalwood oil is the essential oil distilled from the heartwood tissue of mature *Santalum album* trees. Indian sandalwood oil (ISO) is composed over 125 compounds, two main sesquiterpene alcohols namely alpha and beta santalols are responsible for the known bioactivities and distinct aroma [8, 9].

ISO has been widely used in perfumery but rarely used as an active ingredient in modern cosmetics due to the lack of scientific information to substantiate the benefits. There are several studies conducted to evaluate pharmacological properties of ISO such as anti-inflammatory, anti-cancer, anti-microbial etc. [10]. Several in-vitro studies have identified the anti-inflammatory action of ISO on human keratinocytes by inhibiting phosphodiesterase enzyme and tyrosinase inhibition activity [11,12]. This

article presents some of the studies our research team has undertaken to establish the adaptogen properties of ISO [13, 14]. Antioxidant capacity against environmental stressors will be established through *in-vitro* study on human keratinocytes. Adaptogen ability of Indian sandalwood will be studied on a controlled clinical study by measuring the oxidised squalene as an indication of skin protection.

Methodology

Indian sandalwood oil was provided by Quintis Sandalwood (Perth, WA, Australia). This oil was steam distilled from the aromatic heartwood of the tree Santalum album, which was grown in plantations located in Kununurra, Western Australia. The oil was tested and confirmed to comply with ISO 3518:2002. Quercetin and α-tocopherol (Sigma Aldrich, USA) were used as positive controls. The investigational products for clinical study consisted of five different formulae, notably 10% w/w, 1% w/w and 0.1% w/w Indian sandalwood oil in caprylic triglycerides, a vehicle control caprylic triglycerides and a positive control α-tocopherol (0.5% w/w) in caprylic triglycerides.

Each blue light lamp (412 nm and 450 nm) consisted of 10 identical LEDs (Honglitronic, Guangzhou, China) emitting continuous visible radiation embedded in a reflector, which was covered by a transparent glass window. A single peak with a maximum wavelength of either 412 nm or 450 nm could be observed for the lamps. The aperture on the light source was 4.5 cm × 4.5 cm. The array at the surface of exposure was approximately 10 cm × 10 cm, at an approximate distance of 5 cm from the light source. The time of exposure was adjusted to ensure that 1 J/cm2 of blue light was delivered to the investigational site. In clinical studies the distance between the blue light lamps and the exposed zones was adjusted to ensure that volunteers were exposed to 60 J/cm2 of blue light for 30 minutes [15].

A transparent exposure chamber designed to accommodate the well plates and a cigarette connected to an air pump were used (Tarsons, Kolkata, India). During 30 min exposure, three cigarettes were used. The cigarette was connected to a pump that mimics the aspiration of the smoker, and the smoke released in the chamber corresponds to exhaled smoke. Three standard cigarettes were used in the 30 min exposure time [16].

Particulate matter (NIST SRM 1649b) was exposed to subjects' skin on the upper back at a specific concentration of 100 µg/m3 for a duration of 2 h within specially designed cylindrical cups (Ø: 5 cm; height: 3 cm) fitted onto the skin by double-sided tapes. Each cup had one inlet for the incoming particulate matter, at a flow rate of 500 mL/min, and for two other outlets. This detector displayed the particle size distribution (PM1, PM2.5, PM10 and others) as well as the total particle count in the air mixture [13].

Human immortalised keratinocyte cell line HaCaT (ATCC, USA) was cultured in Dulbecco's Modified Eagle's Medium was used for the cultures as per the method specified on Francois-Newton *et al*. 2021 [13].

In vitro cell viability evaluation on the HaCaT cell line was performed with different concentrations of Indian sandalwood oil in ethanol for 48 hrs as described by the method specified on Francois-Newton *et al*. 2021 [13].

Based on the data obtained from the cytotoxicity and solubility tests, the optimal concentration of Indian sandalwood oil was determined by testing the Indian sandalwood oil at eight different concentrations: namely, 0.2%, 0.1%, 0.07%, 0.05%, 0.025%, 0.01%, 0.005%, and 0.001%. As a positive control, quercetin (Sigma-Aldrich, St. Louis, MO, USA) was also tested in this assay at six different concentrations of 0.0075%, 0.00375%, 0.001875%, 0.0009375%, 0.00047%, and 0.00023%. Then, the HaCaT cells were treated with 1 mm of the non-fluorescent probe, 20 ,70 -dichlorofluorescein diacetate (DCFH-DA) (Sigma, St. Louis, MO, USA) and the oxidation reaction was started by the addition of 600 µM AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride) (Sigma, St. Louis, MO, USA).

Approximately 1 × 105 HaCaT cells were seeded per well in different 24-well plates. After the adaptation incubation period of 16 h at 37 ◦C, the Indian sandalwood oil was added to the cells at three different concentrations (0.2%, 0.1%, and 0.05%) for 24 h at 37 ◦C and supplemented with 5% CO2. The positive control alpha-tocopherol (vitamin E) (Sigma-Aldrich, St. Louis, MO, USA) was also tested at three different concentrations: namely, 15 units (corresponding to 2%), 7.5 units (corresponding to 1%), and 3.75 units (corresponding to 0.5%). Then, the cells were treated with DCFH-DA (Sigma, St. Louis, MI, USA) for three hours at 37 ◦C and 5% CO2. After the DCFH-DA treatment, the cells were washed three times with 1X PBS. Then, the 96-well plates were exposed to 412 nm and 450 nm blue light at 1J/cm2 or cigarette smoke as an environmental stressor and left unexposed in a dark environment as a control.

The clinical study was conducted in compliance with the protocol, current internal procedures and in the spirit of ICH Topic E6 (R2). This study was conducted as a monocentric, controlled, randomized, double-blinded, intraindividual comparative trial at the clinical facility at the CIDP Mauritius. This study was submitted to the Fortis-Darné Clinique Independent Ethics Committee (IEC) with the study code 2021CMCL059 and was approved on the 29 January 2021. A clinical examination of the back of each subject was performed by the investigator at the baseline, and on each subsequent visit for the assessment of local tolerance and the reporting of adverse events.

A total of 22 healthy subjects between 18 and 65 years old were recruited as subjects for this study. The main exclusion criteria were as follows: pregnancy, breastfeeding or planning a pregnancy, any hypersensitivity or known contact allergies and major skin conditions. The subjects were instructed to maintain their current hygiene and cosmetic routine and not to do any sunbathing, which may interfere with the study assessments.

The study duration was eleven days, where subjects attended a baseline visit at D0 for acclimatization and the application of the product on seven demarcated investigational zones of 3 cm × 4 cm on the upper back. From D1 to D6, the subjects returned to the investigation centre for the application of 2 mg/cm2 of the product on the investigational zones. On D7, D8 and D9, the application of the product was carried out on the defined zones, followed by an adaptation period of 15 min at 22–23 ◦C with a humidity of 50–60%. An exposure of the exposed zones to ambient dust (NIST SRM 1649b) was done using the CPES, followed by the exposure to blue light at a wavelength of 412 nm. The study ended on D10, whereby product application and exposure were carried out, followed by a sebum sampling of the seven zones one hour after the exposure [11].

The zone of interest is well demarcated with an area of 3 cm^2. The swab is then collected in the cocktail solution and stored at −20 ◦C prior to analysis of the squalene monohydroperoxide by Synelvia Laboratories (Labège, France). Liquid chromatography mass spectrometry analysis for squalene monohydroperoxide was conducted as per the methods described on Francois-Newton et al. (2021) [13].

Experiments were independently repeated in biological triplicate. Qualitative variables were described as the number and percentage of the different response modalities, while the quantitative measurements were summarized using the mean, median, minimum, maximum and the standard deviation. Error bars in the graphical data represent standard estimation of the mean (SEM). A one-way ANOVA was used for the statistical analysis using the software GraphPad Prism Version 7 (GraphPad Software Inc., San Diego, CA, USA), and a statistical significance was claimed when the p-value was lower than 0.01 ($p < 0.01$). All statistical analyses were performed at a 5% significance level using 2-sided tests, except normality tests, conducted at 1% (Shapiro–Wilk test). The SPSS 19.0 (SPSS Inc., Chicago, IL, USA) program was used for statistical analysis purposes.

Results

Prior to the determination of the efficacy of ISO to protect against the oxidative stress induced in HaCaT cells, an MTT assay was performed to establish the optimum concentration of sandalwood oil that would not induce any defect in cell cycle or cell toxicity.

The cell count of HaCaT cells treated for 48 h with ISO was also evaluated. At concentrations of 10%, 2%, 0.6%, 0.5%, 0.4%, and 0.3%, a decrease lower than 70% of the cell count could be observed. However, the cells treated with 0.2% of ISO or less showed cell count results showing more than 70% cell viability. Based on these results, all subsequent efficacy experiments performed in HaCaT cells treatment with ISO were performed at 0.2% as the highest concentration.

Indian sandalwood oil showed antioxidant potential at the five highest concentrations tested (0.2%, 0.1%, 0.07%, 0.05%, and 0.025%). The antioxidant potency of ISO was equivalent to what was observed with the three highest concentrations of the positive control quercetin. These results suggested that at a concentration of 0.2%, ISO has an antioxidant activity that was as potent as the antioxidant activity of the positive control quercetin at 0.0075%. IC_{50} for Indian sandalwood oil was found to be 0.03% and 0.002% for quercetin.

A sharp induction in the levels or ROS can be observed basally when the untreated HaCaT cells were exposed to the stressors: blue light at 412 nm, blue light at 450 nm, and cigarette smoke (Figure 1). When the HaCaT cells were treated with the three concentrations of ISO (0.05%, 0.1% and 0.2%), a significant reduction in the oxidative stress induced by either blue light (412 nm or 450 nm) or cigarette smoke could be observed. Indeed, 66% ($p < 0.0001$), 73% ($p < 0.0001$), and 76% ($p < 0.0001$) decreases in the level of ROS could be observed in cells treated with three concentrations of ISO, respectively prior to exposure to blue light at 412 nm. In cells exposed to blue light at 450 nm, a similar trend could be observed where 60% ($p = 0.0012$), 68% ($p = 0.0005$), and 75 % ($p = 0.0002$) decreases could be observed for similar concentrations of ISO, respectively. Moreover, in HaCaT cells that were exposed to cigarette smoke, ISO was observed to significantly protect against the ROS induced, although the decrease was more modest in comparison to blue light at 412 nm and 450 nm. Thus, at the highest concentration (0.2%) of ISO, only a 28% ($p = 0.001$) decrease in the level of ROS was detected. All three concentrations (0.5%, 1%, and 2%) of the positive control, α-tocopherol, could significantly reduce the ROS induced by blue light 450 nm, only the two highest concentrations of alpha-tocopherol (1% and 2%) could induce a protective effect against the levels of ROS induced by cigarette smoke when compared to the untreated samples(Figure 1). However, none of the three concentrations tested for alpha-tocopherol could significantly decrease the ROS induced by blue light 412 nm. Interestingly, in HaCaT cells treated with the highest concentration of alpha-tocopherol prior to blue light 412 nm exposure, an increase in the levels of ROS can be compared to untreated but exposed cells.

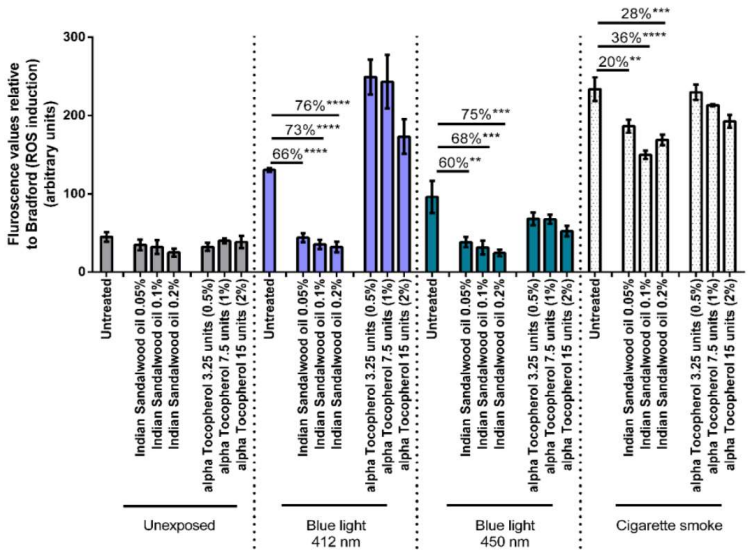

Figure 1: ROS inducement by Blue light 412nm, 450nm and cigarette smoke in HaCaT cells treated with three concentrations of ISO and three concentrations of α-tocopherol. The values represent the fluorescence relative to the Bradford's vale. *p* value **<0.01; ***<0.001; ****<0.0001.

Protective effect of the products was evaluated by measuring the level of the oxidized form of squalene (squalene monohydroperoxide; SQOOH). A four-fold increase in the level of SQOOH was reported in the nontreated exposed zone when compared to the nontreated nonexposed zone. The level of SQOOH collected by the vehicle control following an exposure to the stress was as high as the nontreated and exposed zone, which indicated that the vehicle control does not offer a level of protection against the oxidative stress induced by blue light and ambient dust. A dose-dependent decrease in the levels of SQOOH was reported on the zones treated with the ISO formulations. The zones treated with the positive control, 0.5% α-tocopherol, and exposed showed a lower amount of SQOOH when compared to the exposed zones that were either left untreated or treated with the vehicle control (Figure 2).

Zone comparison also revealed significant differences when the nontreated exposed zone was compared to the 1% and 10% ISO and the 0.5% α-tocopherol ($p < 0.001$). A significant decrease was obtained for the 1% and 10% sandalwood and the 0.5% α-tocopherol when compared to the vehicle control. No significant change in the level of SQOOH was reported in the zone treated with the placebo compared to the exposed untreated zone (Figure 2).

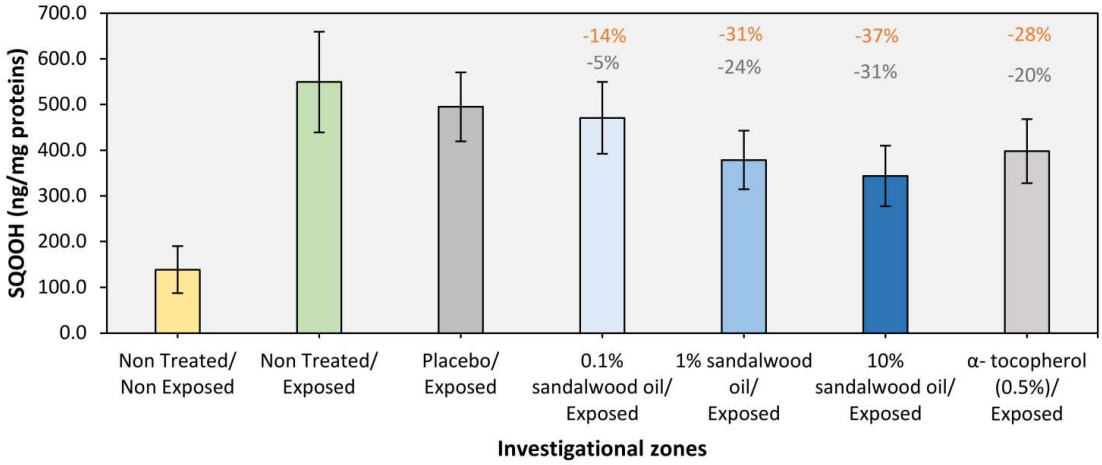

Figure 2:Concentration of SQOOH (ng/ mg of protein) collected on each zone subjected to ambient dust and blue light stress. Percentage variation from untreated exposed zones denoted in orange, exposed zones are denoted in grey.

Discussion and Conclusion

Indian sandalwood oil was found to have low to negligible effect on cell viability and proliferation on human keratinocyte HaCaT cells as evaluated by MTT assay indicated by DCFH-DA. A preliminary cellular antioxidant study conducted on HaCaT cells found that ISO shows an IC_{50} of 0.03% when compared to 0.002% quercetin in neutralising ROS generated by AAPH. Indian sandalwood oil showed lower activity when compared to quercetin in neutralising externally generated ROS. This study has identified that ISO comprised of sesquiterpene alcohols has a different mechanism of action to polyphenolic quercetin in antioxidant activity. However, the higher concentrations of ISO have shown an antioxidant efficacy similar to positive control. Therefore, intracellular antioxidant potential of ISO to protect skin from external stressors were further investigated.

Positive control of choice for intracellular study was lipophilic antioxidant α-tocopherol, which is widely used in personal care products for skin protection [17]. Indian sandalwood oil has shown significant activity than α-tocopherol especially in protecting keratinocytes from solar blue light ($p < 0.0001$) also digital blue light ($p < 0.002$). However, both ISO and α-tocopherol have shown similar activity in protecting skin from pollution simulant cigarette smoke. Indian sandalwood oil in concentrations of 0.05%, 0.1% and 0.2% found to be effective in exerting an in-vitro bioactivity in human keratinocytes. The established mechanism of action for α-tocopherol is by neutralising ROS, while ISO is not rich of oxygen quenching molecules, it is hypothesized that the ISO could upregulate keratinocyte's self-antioxidation mechanism [18,19].

Controlled clinical study conducted on volunteers have resonate the findings of the *in-vitro* study finding that 0.1%, 1% and 10% of Indian sandalwood oil in simple dermal application has significant effect of protecting skin from pollution simulating ambient dust and blue light. Highest protection was reported for 10% ISO with a 31% change from the placebo, at 0.1% ISO there was a numerical difference but no significant statistical significance to the placebo. Positive control, α-tocopherol performed similar to when comparing the data for ISO at 1% and 10%.

Pollution and blue light are seen as significant contributors for skin damage. Initial step of this damage could be the *in-situ* oxidation of epidermis mediated by ROS. Products that can protect the skin from further damage act initially as antioxidants. It is interesting to observe that ISO did not act as a ROS neutraliser but was effective in intracellular scavenging of ROS. This activity is important for a sustained antioxidant activity on the skin [20].

Adaptogen properties of Indian sandalwood oil has not been previously studied for its potential application in cosmetic products. Current study has evaluated the intracellular antioxidant properties of ISO in protecting keratinocytes when stressed with pollution and blue light, this was further tested on human skin to establish the antioxidant potential. ISO has exhibited its adaptogen potential by protecting skin from oxidation thus blocking the onset of a cascade of biological reactions causing further damage to skin causing pigmentation, inflammation, and aging. Indian sandalwood is a multipurpose ingredient with aroma, fixative properties, and cultural significance; newly evaluated apoptogenic properties would establish Indian sandalwood oil as an active cosmetic ingredient.

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Conflict of Interest

The authors declare no conflict of interest.

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