The real active ingredient from Santalum Album: efficacy studies in an anti-hair loss protocol

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Abstract

Heavy hair loss and reduced hair density represent for all humans problematic and impacting conditions on daily life. Hair thinning is commonly associated with men, but also women can suffer from this condition; Causes are to be found in genetics factors, hormone imbalance, stress and exogenous factors causing inflammation. The use of natural *Santalum album* (sandalwood) oil for fighting hair loss is controversial, as its efficacy has not yet been demonstrated *in-vivo*. The aim of our study is to investigate the anti-hair loss property of sandalwood oil through an *in-vivo* test. The pure sandalwood oil was introduced into a hydro-alcoholic lotion to be daily applied to the scalp for three months, at two different levels (0.1 and 0.5% w/w) on volunteers (both gender) suffering from androgenic alopecia.

The evaluation and quantification of results was performed by the photo-trichogram method.

The efficacy of the oil is proven by statistically significant improvements in percentage of anagen hair (increase) and percentage of telogen hair (decrease) with lotion at 0.5% of Sandalwood Oil.

Despite the remarkable cost due to its unique preciousness, Santalum album essential oil seems a very promising material for reestablishing the scalp equilibrium.

Key words: sandalwood oil, hair-loss, hair growth, santalol, photo-trichogram

1. Introduction

Human hair plays an important role in social communication, in addition to protective function. Hair life cycle involves the alternation of three phases: anagen (growth), catagen (rest), and telogen (fall). The average duration of this cycle varies from 2 to 7 years.

Baldness or androgenetic alopecia (AGA) is defined as a slow transformation of large scalp terminal hair follicles to shorter and thinner hair with a much shorter anagen phase (hair miniaturization).

AGA directly distresses self-confidence affecting the individual's quality of life and hits 50% of men and 20% of women at some stage in their lives: men typically present it with recession of the hairline at the temples and hair loss at the vertex, while women normally have diffuse thinning across the entire upper part of the scalp [1], [2].

Besides genetic factors and age, the main aspects that adversely affect hair growth are hormone imbalance (high testosterone, high cortisol, unbalanced thyroid hormone), stress and exogenous factors causing inflammation (pollution, sun exposure, excessive use of hair bleaching or dyeing products).

The subsequent steps of AGA occur in the bulbs of the frontal and vertex region. Anagen phase time decreases, and consequently the telogen phase begins earlier. The resulting stem will be thinner and shorter (vellus). Over a period of many months or years, earlier bulbs death leads to the definitive disappearance of hair.

This process does not occur uniformly and is described in the 'stages' of the Hamilton Scale and the Norwood Scale.

Generally, in men the hair-loss process starts in the frontal region, because 5α-reductase is more active there, so more DHT is concentrated there; later the same happens in the vertex; finally, baldness affects the entire upper part of the head.

The mechanisms just described add up to the fact that the nourishment of the bulbs is a critical factor. Indeed, the most peripheral area of blood circulation is the scalp, and in this part of the body is the most prone to vessel atrophy.

Treatment for hair loss is different from other dermatology disorders, for instance psoriasis and atopic dermatitis, as it will need a longer period of treatment until significant improvement has been noticed. Ideal treatment for hair loss is a combination of 5α-reductase inhibitors with hair growth promoter substances.

Anti-androgen mechanisms are generally categorized into three actions: either inhibit or trap DHT or inhibit 5α-reductase or blocking androgen receptors [3].

From the literature it has been shown that human hair follicles can engage in olfactory receptor-dependent chemo-sensation, suggesting that olfactory receptors may serve as a target in hair loss therapy.

In human body, olfactory receptors are not only expressed by the nasal epithelium, but also by different cell types.

Specifically, the epithelium of human hair follicles, particularly the outer root sheath, prominently expresses the olfactory receptor OR2AT4 in situ, namely in basal keratinocytes layer [4]. The specific stimulation of OR2AT4 by a synthetic sandalwood odorant called Sandalore promotes proliferation and migration of keratinocytes and prolongs human hair growth ex vivo (organ-cultured human scalp hair follicles); in particular, decreasing apoptosis and increasing production of the anagen-prolonging growth factor IGF-1 are observed. Concurrently, silencing of OR2AT4 inhibits hair growth. This supports that human hair follicle diseases treatments with selected odorants may be possible, since olfactory receptors-mediated signaling regulates human hair follicles and keratinocytes biology in situ and OR2AT4 stimulation by endogenous ligands is required to down-regulate keratinocytes apoptosis and, consequently, the catagen phase [5].

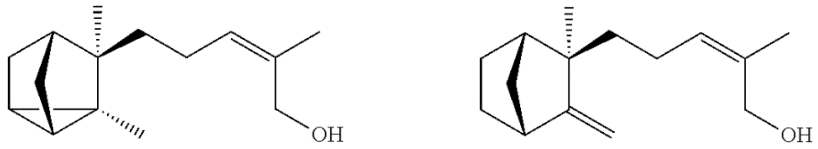
Using natural sandalwood oil to counteract hair loss is still controversial as there are no studies in the literature demonstrating its effectiveness in vivo.

Santalum album (sandalwood) is a medium-sized (up to 9 meters) hardwood tree belonging to the Santalaceae family. It is a hemi parasitic evergreen tree that needs a host plant to grow: in fact, it combines with the roots of host plants to get nourishment and water. The tree also needs special conditions to thrive: fertile, well-draining soil, sufficient irrigation during the dry season, a subtropical climate and, ideally, a protected location that offers shelter from storms and bad weather. The tree difficult growing conditions explain the high cost of the raw material.

Santalum album oil (CAS # 8006-87-9) is the related volatile essential oil derived from the rich heartwood of the tree. The most common method of extracting is steam distillation of the commutated dried wood from the trunk and roots. Steam causes the small secretory structures in the wood to explode and release the precious essential oil. The oil has a woody, exotic, subtle and persistent odour; its colour ranges from pale yellow to golden [6].

The oil is made up of more than 100 components; the major components are sesquiterpenoid alcohols ($C_{15}H_{24}O$): Z-α-santalol (41-55%) and Z-β-santalol (16-24%) (fig.1 and 2) [7].

Figure 1: Chemical structure of Z-α-santalol (left) and Z-β-santalol (right)



Sandalwood is deeply rooted in the cultures of India, Sri Lanka and the Middle East; in these locations, it is considered one of the most extraordinary and valuable resources and has been used for more than 3000 years because of its woody, long-lasting fragrance and excellent carrier properties [8]; equally unique are its dermatological and medical properties (anti-inflammatory [9], antioxidant [10] and antimicrobial properties [11]). These are the reasons that make this valuable raw material an interesting active source of phytochemicals suitable for health and personal care applications.
The aim of our study is to investigate the anti-hair loss property of sandalwood oil through an in-vivo test on volunteers suffering from androgenic alopecia. Pure sandalwood oil was introduced into a hydro-alcoholic lotion to be daily applied to the scalp for 3 months.

A non-invasive and reproducible way to quantify changes of hair density and hair growth parameters has been performed applying the photo-trichogram method. It allowed to evaluate changes in percentage of anagen and telogen hair. Treatment efficacy and pleasantness have been rated by filling a questionnaire.
The instrumental and clinical analysis performed at the end of the treatment showed very striking results. A statistically significant increase in the percentage of anagen hair and a decrease in the percentage of telogen hair were observed. This result leads to the conclusion that Santalum album essential oil seems to be a very promising material for restoring scalp balance.

2. Materials and Methods

2.1. Sandalwood oil

The essential oil used for this study is a pure Sandalwood oil (CAS Number 8006-87-9/84787-70-2) supplied by Alpha Santanol Pty Ltd (17-21 Coulson Way, WA 6155) and obtained from plantations of East Indian Sandalwood (*Santalum album*) in Western Australia by steam distillation. It is a clear and slightly viscous liquid, with a golden

yellow colour. It is characterized by several constituents; the main ones, in terms of quantity and activity, are Z-alpha-santalol (41 to 55%) and Z-beta-santalol (16 to 24%).

2.1.1. Characterization of Sandalwood oil

In order to extend the knowledge about the composition of Santalum album oil, thermostability and compatibility with the solvent of the lotion used in this study, GC-MS analysis were performed on three samples:

- Sample 1: pure sandalwood oil stored at T = 25°C;
- Sample 2: pure sandalwood oil stored for two months at T = 42.5°C;
- Sample 3: 2% m/m sandalwood oil solution in ethanol (oil-ethanol ratio of the 0.5% lotion used in this study), stored at T = 25°C.

10 mg of sandalwood oil was diluted in 1.5 mL of ethyl acetate. To this solution, 20 mg of bis(trimethylsilyl)trifluoroacetamide (containing 1% trimethylchlorosilane) was added as a derivatizing agent for the alcohol groups. Sample left to age in ethanol was previously concentrated under reduced pressure in order to remove most of the ethanol before derivatization.

2.1.2. GC-MS method

The pretreated sample was injected onto Clarus 560 S PerkinElmer system. The column used was Elite-5MS 30.0m x 250µm, 0.25µm df, coated with 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane, operated with the following oven temperature program: 70°C, held for 10 min, rising at 2°C/min to 220°C, held for 5 min; total time of the experiment: 90 min; injection volume: 1.0µl; carrier gas: helium. Mass spectrometer parameters were as follows: ionization mode, EI (electron ionization); acquisition mass range, 50–400m/z; solvent delay time, 4 min. For the identification of compounds, a GC–MS database was used.

2.2. Hair lotion

On the basis of the analyses reported in the previous section, to evaluate the effectiveness of sandalwood oil in preventing hair loss, the ingredient was introduced in a hydro-alcoholic lotion at two different percentages: 0.1% and 0.5% w/w.

Below the INCI list of the lotion: Aqua, Alcohol Denat., Pentylene Glycol, Ethoxydiglycol, PEG-40 Hydrogenated Castor Oil, Inositol, Santalum Album Oil,

Glucosyl Hesperidin, Menthyl Nicotinate, Betaine, Trisodium Ethylenediamine Disuccinate, Citric Acid, Polyquaternium-11, Helianthus Annuus Seed Oil, PEG-8, Tocopherol, Rosmarinus Officinalis Leaf Extract, Ascorbyl Palmitate, Ascorbic Acid.
The addition of some components was necessary to ensure oxidation stability and adequate skin penetrating power to sandalwood oil.
The pH value of the formulas was 4.8 ± 0.2.

2.3. Phototrichogram analysis

The efficacy in preventing the hair loss of the two lotions containing Sandalwood oil at 0.1% and 0.5% was evaluated by instrumental analysis.
The study is carried out on 20 subjects suffering from androgenic alopecia: II–IV stage of Norwood-Hamilton's scale for males and I–II stage of Ludwig's scale for females.
During the study, subjects are instructed to wash their hair using their current hair care regimen and not to apply the tested product on any other site than the prescribed ones.
For the whole duration of the test, the subjects must not use products for preventing the hair loss different from the tested ones, either topic or systemic.
The study was carried out in a temperature and humidity - controlled room (24 ± 2 °C; 50 ± 10 % R.H.).
10 subjects applied the lotion at 0.5% Sandalwood Oil, while the other 10 subjects applied the lotion at 0.1% Sandalwood Oil. Both the lotions were applied once a day (2 ml) for 3 months on the whole scalp. The products (0.5% and 0.1% lotions) were assigned to the subjects following a randomized treatment schedule. The assignment of subject number and subsequent placement on the randomization chart were made in order of appearance at the study centre on the first day.
Volunteers were asked to wash their hair 48 hours before each visit and to refrain from the application of any styling products.

2.3.1. Phototrichograms method

The phototrichogram technique is based on taking a photograph of a defined hair clipped area of the scalp after a period of time long enough to permit the evaluation of the growth of the hair (usually between 24 and 72 hours). The number and the type of hair (anagen or telogen) in the area are determined by means of the analysis of the photographs.
Hair in the anagen phase is in growth phase, while the follicles of hair in telogen phase are almost quiescent. The hair growth rate is about 0.35 mm per day.

By means of the software Trichoscan Professional, the following parameters were calculated at each control time, in an area of 0.728 cm^2 within the hair clipped site on the scalp:

- the percentage of anagen hair (length > 0.65 mm) in 0.728 cm^2;
- the percentage of telogen hair (length ≤ 0.65 mm) in 0.728 cm^2.

2.3.2. Mathematical Elaboration

Mean values, standard deviations and variations were calculated for each set of values.
Following the results of the normality test (Kolmogorov-Smirnov test) the instrumental data of each group (T0 and T3months) were statistically compared by means of t-test for parametric and dependent data while the clinical scores (T0 and T3months) were compared by means of Wilcoxon test for non-parametric and dependent data.
Following the results of the normality test (Kolmogorov-Smirnov test) the variations of the two groups of the instrumental data (T3months - T0) were statistically compared by means of t-test for parametric and independent data while the clinical scores (T3months - T0) were compared by means of Mann-Whitney U test for non-parametric and dependent data.
The differences between the groups of values were considered significant when the probability p was < 0.05.

3. Results

Below are the results obtained in each test performed.

3.1. Characterization of Sandalwood oil

As can be seen from the multitude of peaks, the oil is made up of many constituents. The peaks with the largest area are related to Z- α-santalol (RT=53.02 min, %A =48.4-52%) and Z- β-santalol (RT = 55.01 min, %A = 18,2-24,4%); the other much smaller peaks are related to sesquiterpene and sesquiterpenol derivatives and their isomers. Note that only the 15 components that could be detected are listed in the table; the oil also consists of many other compounds, but these were not detected as they were present at an extremely low concentration. By comparing the chromatographic profiles associated with the two different temperature conditions, it can be seen that they fit perfectly on top of each other (there are no significant differences in terms of retention times, number and integral of peaks, especially for the two main components).

This demonstrates the thermostability of the oil and its main components and promotes its use in products that are exposed to such temperatures. Even in case of sample 3, no significant differences from the chromatographic profile of pure sandalwood oil were revealed, highlighting its stability in EtOH. This result was essential to assure the stability of the oil in the hydroalcoholic lotion used on volunteers.

Graph 1: GCMS profiles of samples 1,2 and 3. (See table 1 for peak identities)

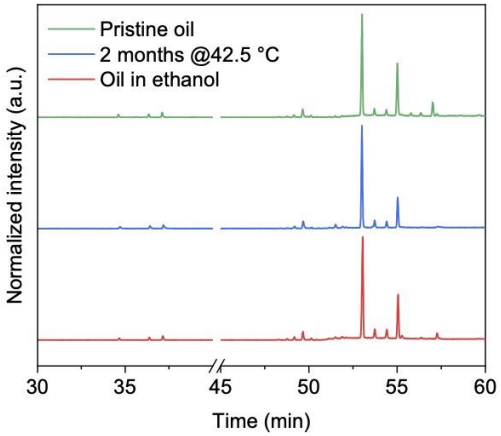


Table 1: Chemical composition of Santalum Album oil in samples 1 (S1),2 (S2) and 3 (S3).

			GC-MS peak integral			*% Area*		
No.	*INCI name*	*RT (min)*	*S1*	*S2*	*S3*	*S1*	*S2*	*S3*
1	α-santalene	34.62	881456	531049	1147943	1.4	1.4	1.0
2	epi-β-santalene	36.35	986565	644793	1474433	1.6	1.8	1.3
3	β-santalene	37.11	1528827	1053741	2356824	2.5	2.9	2.4
4	Epi-cyclosantalal	48.80	270162	130378	789945	0.4	0.4	0.7
5	cyclosantalal	49.19	719041	506944	1809417	1.2	1.4	1.6
6	Z-α-santalal	49.72	2673211	1875213	4945816	4.3	5.1	4.2
7	α-curcumene	50.12	485882	183960	985653	0.8	0.5	0.8
8	β-bisabolene	51.13	158554	164298	468730	0.3	0.4	0.4
9	α-bisabolol	51.51	344972	903656	826822	0.6	2.5	0.8
10	Trans-α-santalol	51.92	420609	280708	859076	0.7	0.8	0.2
11	**Z-α-santalol**	**53.02**	**30317988**	**21022136**	**60646736**	**48.4**	**54.4**	**52.0**
12	Z-α-trans-bergamotol	53.73	2238257	1859892	5490010	3.6	5.1	4.7
13	Z-epi-β-santalol	54.39	1672807	1275305	5457199	2.7	3.5	4.7
14	**Z-β-santalol**	**55.01**	**17360678**	**6717284**	**25300884**	**24.4**	**18.2**	**21.7**
15	cis-lanceol	57.27	4514625	659212	3982155	7.3	1.8	3.4
	TOT		*64573634*	*37808569*	*116541643*	*100*	*100*	*100*

3.2. Phototrichogram Analysis

3.2.1 Percentage of anagen hair in 0.728 cm^2

A statistically significant increase in the mean percentage of anagen hair was recorded for the lotion at 0.50% of Sandalwood Oil after 3 months of treatment.

A non-statistically significant increase in the same parameter was evidenced for the lotion at 0.10% of Sandalwood Oil at the end of treatment.

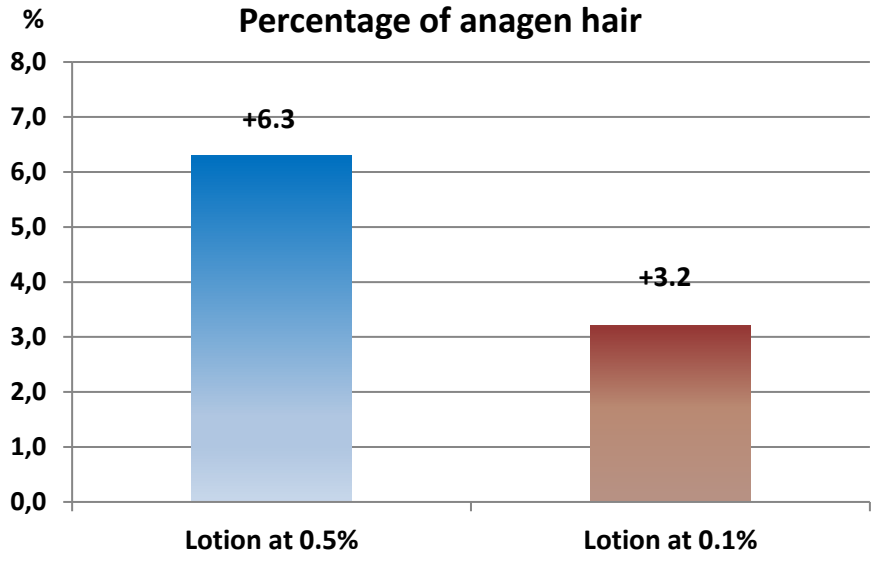
The statistical comparison between the variations of the two groups did not show any significant difference.

Table 2: mean values, standard deviations, variations and statistical comparisons.

	T_0	$T_{3months}$	$T_{3months} - T_0$	T_0 vs $T_{3months}$ p-level
LOTION at 0.5%	mean 50.5% *std. dev. 13.4*	mean 56.8% *std. dev. 10.9*	+6.3%	**$p < 0.05$**
LOTION at 0.1%	mean 61.5% *std. dev. 6.7*	mean 64.7% *std. dev. 7.9*	+3.2%	$p > 0.05$

($T_{3months} - T_0$) **LOTION at 0.5%** vs ($T_{3months} - T_0$) **LOTION at 0.1%**	$p > 0.05$

Graph 2: percentage of anagen hair variations of the two groups.



3.2.2 Percentage of telogen hair in 0.728 cm^2

A statistically significant decrease in the mean percentage of telogen hair was recorded after 3 months of treatment for the lotion at 0.50% of Sandalwood Oil.

A non-statistically significant decrease in the same parameter for the lotion at 0.10% of Sandalwood Oil.

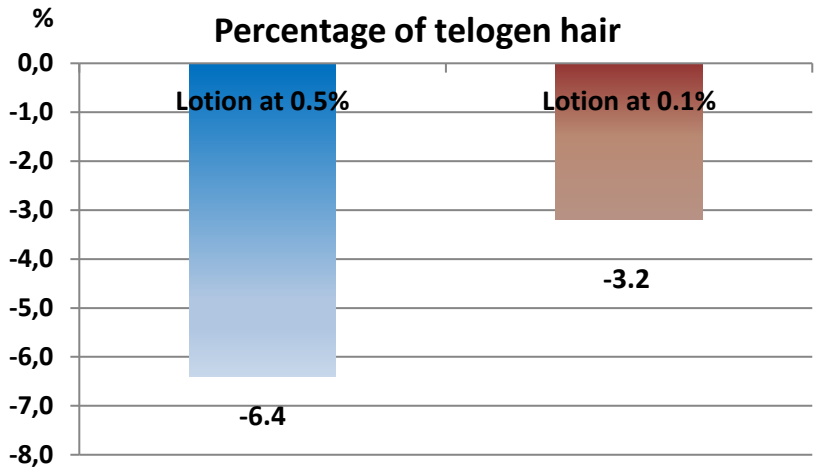
The statistical comparison between the variations of the two groups did not show any significant difference.

Table 3: mean values, standard deviations, variations and statistical comparisons.

	T_0	$T_{3months}$	$T_{3months} - T_0$	T_0 vs $T_{3months}$ p-level
LOTION at 0.5%	mean 49.6% *std. dev. 13.4*	mean 43.2% *std. dev. 10.9*	-6.4%	**$p < 0.05$**
LOTION at 0.1%	mean 38.5% *std. dev. 6.7*	mean 35.3% *std. dev. 7.9*	-3.2%	$p > 0.05$

($T_{3months} - T_0$) **LOTION at 0.5%** vs ($T_{3months} - T_0$) **LOTION at 0.1%**	$p > 0.05$

Graph 3: percentage of telogen hair variations of the two groups.



Some examples of phototrichogram images made by FotoFinder Dermoscope and Trichoscan Professional Ver. 2.0 are reported below.

Figure 2.1: subject 17, T 0, lotion 0.5%

Figure 2.2: subject 17, T 3 months, lotion 0.5%

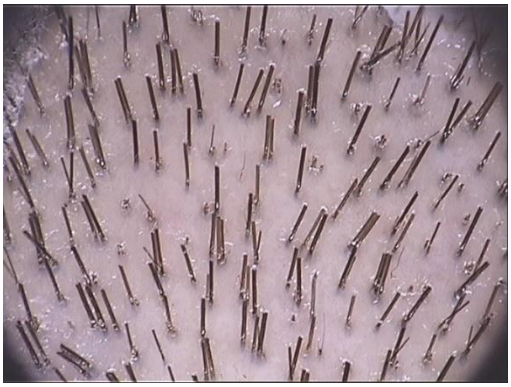


Figure 3.1: subject 11, T 0, lotion 0.1%

Figure 3.2: subject 11, T 3 months, lotion 0.1%

4. Discussion

The effectiveness in terms of hair loss reduction has been widely demonstrated for synthetic derivatives of sandalwood oil, able to interact with the olfactory OR2AT4 receptors located in the epithelium of human hair follicles, promoting hair growth by decreasing apoptosis and increasing production of IGF-1 in the outer root sheath. Although Indian folk medicine and traditional Chinese medicine report, among the various benefits associated with pure sandalwood oil, a stimulating action on hair growth, to date no clear evidence of this activity was available.

In this study, a scientific approach was applied in order to demonstrate the effectiveness of pure sandalwood oil in preventing hair loss. For this purpose, the essential oil was introduced in a hydro-alcoholic solution, containing stabilizing ingredients, the oil being

rich in potentially oxidizable substances, and ingredients to promote penetration into the skin.

Through instrumental, clinical and subjective analysis, and the subsequent statistical re-elaboration, it has been shown that the use of a 0.5% sandalwood oil lotion for 3 months results in a statistically significant increase in the percentage of hair in the anagen phase and a statistically significant reduction in the percentage of hair in the telogen phase.

5. Conclusions

This study demonstrated the effectiveness of Sandalwood oil in preventing hair loss when introduced in a hydro-alcoholic solution to be applied topically on the scalp. In particular, at the concentration of 0.5% the lotion has showed a statistically significant increase in the percentage of anagen hair (+6.3%) and a statistically significant decrease in the percentage of telogen hair (-6.4%).

Conflict of Interest Statement. No conflict of interest.

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