A unique Lavandin Absolute bringing skin and wellness benefits.

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1. Introduction

Fragrances are widely used in cosmetic products to give a distinct scent and to guide perception of product performance. The use of fragrances is a daily ritual that can influence emotional states, mood, and social interactions. Nowadays, consumers expect fragrances to go beyond hedonism bringing emotional and skin benefits.

Lavender and Lavandin are well-known plants with purple flowers growing in the Mediterranean region. The genus *Lavandula*, native to Persia, includes more than thirty species, and many hybrids [1]. Lavandin is a hybrid of two wild lavenders: fine lavender (*Lavandula angustifolia Mill.*) and spike lavender (*Lavandula latifolia Medik.*). In France, lavandin and lavender crops are the largest crops of aromatic plants and a symbol of Provence. Lavender and Lavandin species share ethnobotanical properties and produce similar valuable metabolites [1, 2]. Their therapeutic benefits are attributed to their richness and diversity in phytochemical compounds promoting a broad spectrum of biological activities such as antioxidant, anti-inflammatory, analgesic and anxiety-reducing effects [3]. Lavandin aromatic components can be found not only in essential oils but also in extracts such as concretes and absolutes [4].

In the fragrance industry, "absolutes" are natural fragrance extracts usually obtained by extraction using organic solvents. In this study, the **Lavandin Absolute** is obtained with lavandin harvested with the Espieur process, in which only flowered heads are picked to extract the most valuable olfactory molecules. This unique harvesting method, combined with an exclusive proprietary process of extraction, allows the production of an organic absolute, with a characteristic scent close to the genuine lavandin flowers. The **Lavandin Absolute** obtained is enriched in valuable bioactive molecules usually found as minor in traditional hexane absolutes [5]. In-silico and artificial intelligence-based bioinformatics tools allowed to identify potential biological implications of the **Lavandin Absolute**. *In-vitro* results, in fibroblasts and keratinocytes cultures, showed higher expression levels for markers linked to improved skin barrier function and skin hydration, as well as decreased levels of cytokines. Application of the **Lavandin absolute** demonstrated respect for the skin microbiome. Molecular docking simulations on GABA and NMDA receptors helped to understand a potential binding affinity of some characteristic compounds in a biological context. Finally, functional Magnetic Resonance Imaging (fMRI) approaches identified this **Lavandin Absolute** as a valuable opportunity regarding its relaxation benefits. To conclude, our study identified this unique **Lavandin absolute** as a natural fragrance ingredient that can be used in cosmetic formulations to help enhance wellness and skin appearance.

2. Materials and Methods

2.1 Plant material, and extraction of the Lavandin

Plant material and extraction

The lavandin flowers were sourced from the Provence region in France, with the Censo certification for a sustainable and traceable supply chain. It is harvested with the Espieur technique, in which only flowered heads are picked. The **Lavandin Absolute** is obtained with an exclusive process of extraction involving a bio-sourced and renewable organic solvent followed by purification with organic ethyl alcohol.

Characteristics of the ***Lavandin Absolute***

The **Lavandin Absolute**, INCI: LAVANDULA HYBRIDA FLOWER EXTRACT, has a characteristic olfactive description linked to its specific process of obtention. It has a freshly herbaceous and floral scent, like a genuine Lavandin flower. The coumarin and hay-like background brings roundness and long-lastingness.

2.2 Bioinformatics

The predicted protein-target for the **Lavandin Absolute** compounds were retrieved, based on a combination of 2D and 3D structural similarities, and with artificial intelligence-based approaches (machine learning). For gene ontology (GO) enrichment analysis, the five most relevant GO terms of the biological process, molecular function, and cellular component groups were considered. The STRING database (https://string-db.org/) was used to collect and integrate protein-protein interactions. Molecular docking simulations were performed with Autodock Vina 1.2.5, after identifications of binding pocket using a deep learning-based method.

2.3 In-vitro efficacy evaluation

The **Lavandin Absolute** was evaluated on reconstructed skin epidermis by immunohistochemistry of Claudin-1 and Envoplakin (48 hours after application), in primary keratinocytes by qPCR array with probes related to barrier function, and in normal human dermal fibroblasts by ELISA for measurement of levels of hyaluronic acid, and interleukin-6 and 8 (after stimulation with recombinant interleukin-1).

2.4 Microbiota equilibrium co culture assays

The "Respect to the microbiome" evaluation was based on a minimal microbiota perturbation. The **Lavandin Absolute** at 0.1% and 0.01% was applied during 8 hours on a microbial co-culture, and Colony Forming Units (CFU) were calculated for each strain. Facial strains were: *C. acnes, C. xerosis, M. luteus, S. epidermidis, and S. mitis.* Scalp strains were: *A. ursingii, C. tuberculostearicum, C. acnes, S. epidermidis.*

*2.5 functional Magnetic Resonance Imaging evaluation of the **lavandin absolute***

The neuroscientific studies were conducted with functional Magnetic Resonance Imaging. Brain activity was measured by detecting changes in blood oxygenation level (BOLD signal) in response to sensory stimulation associated with the smelling of the **Lavandin Absolute**. The fMRI experimentations followed an Event-Related Design. The panelists were blindfolded with a sleeping mask to avoid visual interference and were simply instructed to smell the scent at the sound signal, no task was required other than smelling. Each ingredient, dissolved at iso-intensity in a non-odorant solvent, was delivered in a random order and repeated three times. Sniff duration was 4s for each scent stimulus, followed by a 16s resting time. Images were acquired on a 3T Philips Achieva scanner, equipped with a 32-channel head coil allowing to measure 300,000 voxels for each sniff. Pre-processing of the data was carried out with Brain-Voyager 20.6. Random group analyses (GLM RFX) were performed, thresholded at $p < 0.05$, uncorrected.

3. Results

Aromatic plants produce a broad variety of metabolites with distinct biological activities and potential applications. The exclusive **Lavandin Absolute** contains higher % of lavandulyl acetate, β-caryophyllene, β-farnesene, γ-cadinene and α-bisabolol compared to a classic absolute obtained by extraction with hexane.

3.1 Gene Ontology (GO) enrichment analysis

To explore the potential biological functions of the **Lavandin Absolute**, GO term enrichment and analysis was performed on protein target prediction. The five most relevant GO terms in the molecular function (MF), biological process (BP) and cellular component (CC) term were retrieved (Figure 1).

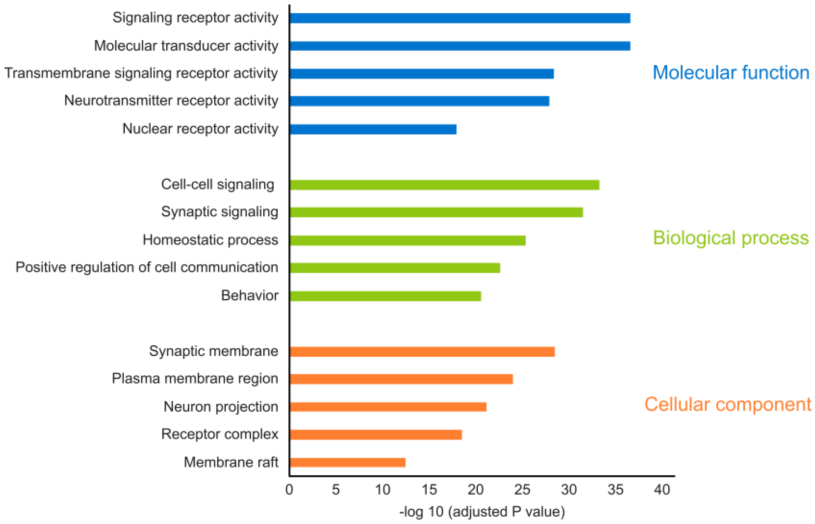

Figure 1. Top five enriched GO functions of the regarding Molecular function (MF), Biological process (BP) and cellular component (CC) terms.

3.2 Functional protein association networks

A protein-protein interaction network was constructed by using the STRING database (Figure 2). The network allowed the Identification of hub genes and transcription factor regulatory network. Four interacting clusters have been identified: Nuclear receptor transcription pathway (red), Neuroactive ligand-receptor interaction (yellow), Synaptic transmission, cholinergic (green), and Synaptic GABAergic (blue). The results of this data mining and integration should help in revealing new mechanisms and targets for the exclusive **Lavandin Absolute**. Protein-Protein Interaction Networks highlighted clusters which could regulate gene expression in cells (Figure 2).

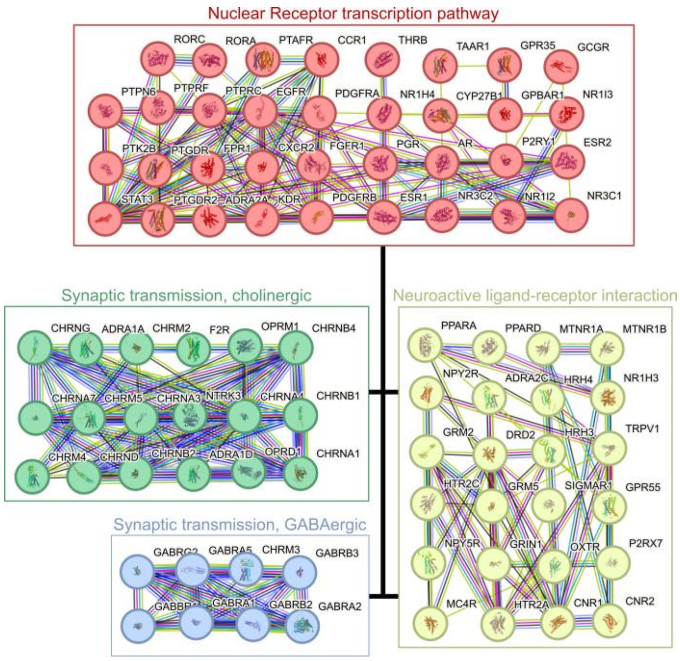

Figure 2. Protein-Protein Interaction Networks. The nodes represent proteins, and the edges represent interactions between proteins. Cluster names: Nuclear Receptor transcription pathway (red),

Neuroactive ligand-receptor interaction (yellow), Synaptic transmission, cholinergic (green), and Synaptic transmission, GABAergic (blue).

3.3 In-vitro evaluation

To obtain a better understanding of the mechanisms promoted by the application of the **Lavandin Absolute** on skin, *in-vitro* efficacy tests were conducted on reconstructed epidermis and primary skin cells. Results showed an increased expression of the skin barrier function markers, and an increased production of hyaluronic acid. In addition, decreased levels in interleukin-6 and 8 in fibroblasts were observed after stimulation by IL-1 (Figure 3).

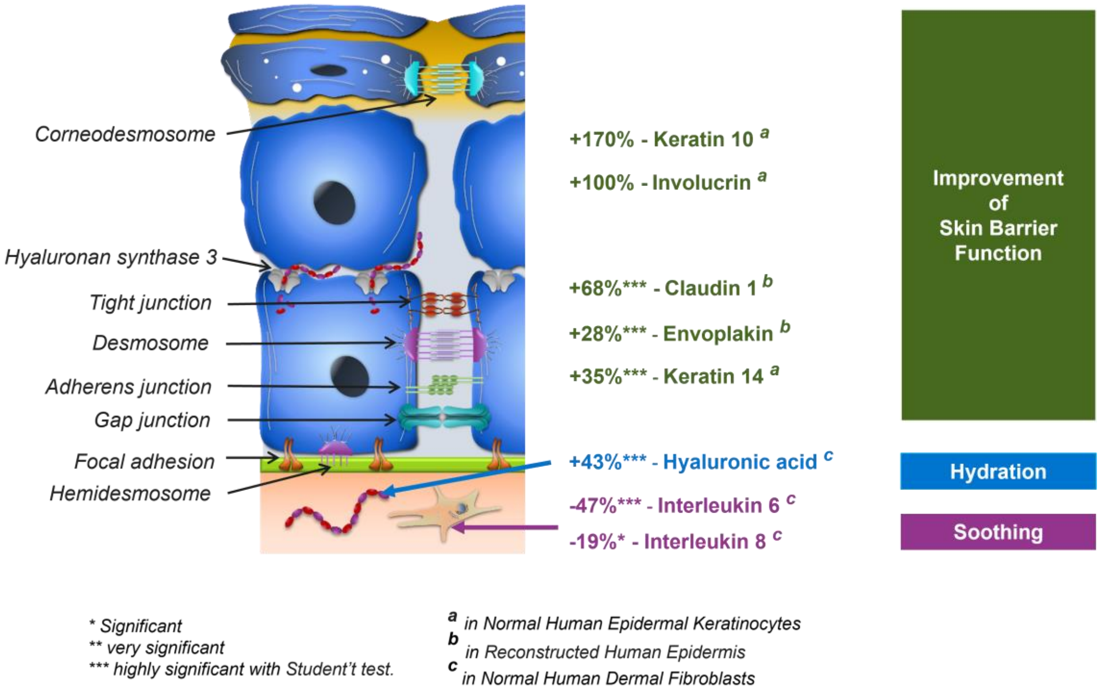

Figure 3. Schematic illustration of the effects of the Lavandin Abslolute on skin barrier function markers (green), hydration markers (blue), and soothing markers (purple).

3.4 Respect to the microbiome

The skin is colonized by microorganisms, most of which are harmless and confer numerous benefits to the host. These microorganisms help the host to maintain a healthy skin barrier, resist to pathogen colonization during wound healing, and modulate the inflammatory response. The application of the **Lavandin Absolute** on bacteria co-cultures did not modify the level and ratio of microorganisms, suggesting respect for the microbiome (Figure 4).

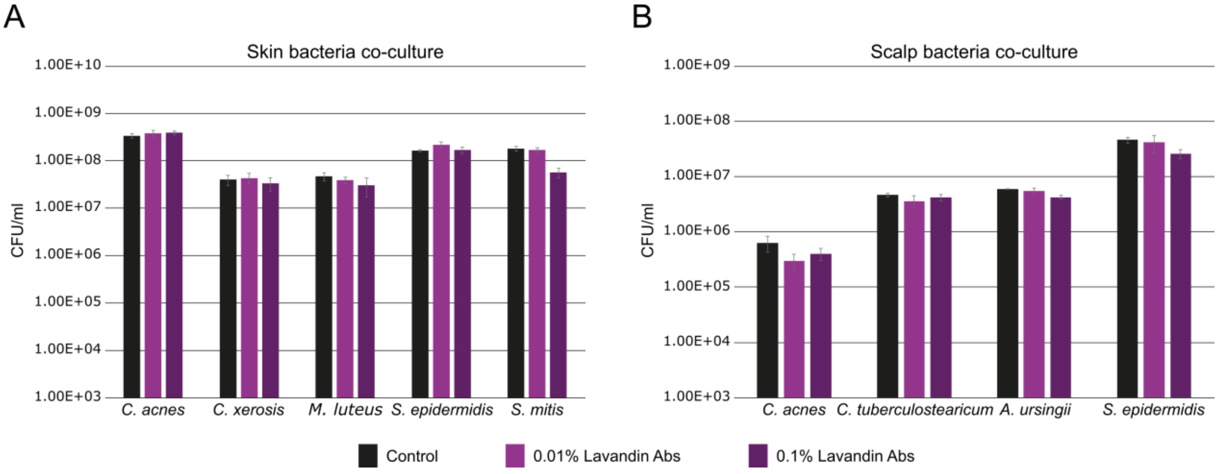

Figure 4. Bacterial cocultures results after application of the Lavandin Absolute. (A) Skin bacteria co-culture. (B) Scalp bacteria co-culture. CFU: colony forming unit.

3.5 Docking molecular simulations on GABAA and NMDA receptors

Docking is commonly used to predict how ligands could bind to protein targets. Linalool is known as one of the compounds linked to the anxiolytic effect of *Lavandula* species. To obtain a better understanding of the implication of other compounds of the **Lavandin absolute**, a docking study was performed on two ionotropic receptors: GABAA, NMDA. The preferential binding pockets were determined by a deep learning-based method. Docking simulations were performed and calculated binding free energies (kcal/mol) were best for three compounds: β-farnesene, γ-cadinene and α-bisabolol (Figure 5).

A

	GABAA receptor α1β2γ2	NMDA receptor
Linalyl acetate	-6.30	-7.13
Linalool	-5.56	-6.90
Lavandulyl acetate	-6.31	-7.34
Caryophyllene	-5.34	-4.00
Borneol	-5.87	-5.04
Camphor	-6.18	-4.55
β-farnesene	-7.15	-8.09
γ-cadinene	-8.30	-9.63
α-bisabolol	-7.47	-8.02

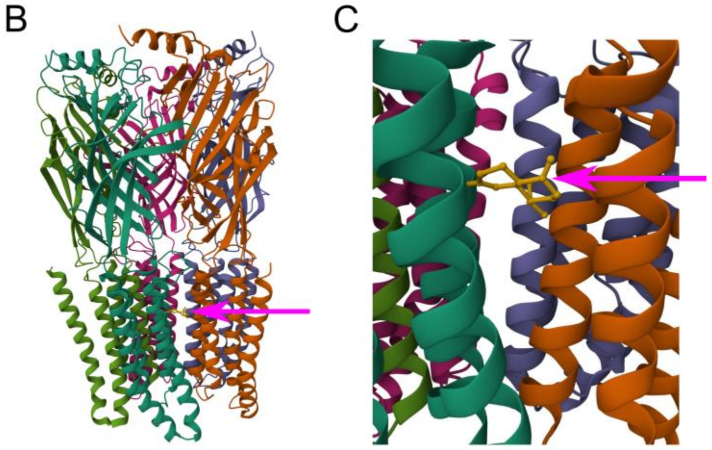

Figure 5. Molecular docking analysis of compounds on GABAA and NMDA receptors. (A) Table showing the binding free energies (kcal/mol) associated with best docking poses. (B) Molecular docking illustration of γ-cadinene best pose on GABAA receptor (PDB: 6X3X). (C) Corresponding zoom representation of γ-cadinene best pose on GABAA receptor. Pink arrows highlight the γ-cadinene (yellow).

3.6 Functional magnetic resonance study

fMRI is a very powerful technique to assess subconscious states and feelings elicited by scents. This technique offers the possibility to visualize the brain networks accurately and thereby evaluate precisely the benefits that a particular scent evokes. **Lavandin Absolute** activated several brain areas that have been categorized as involved in Relaxation and Mindfulness. These brain regions are responsible for self-consciousness, promotion of quietness, regulation of respiration and voluntary movements. Relaxation is an emotional state where arousal is low, tension and stress are at low levels, and positive affect is elevated. On the other hand, Mindfulness is a calm awareness of one's own sensation and experience. Both tend towards the same positive effects on perceived well-being.

4. Discussion

Current societal culture is reviving the practice of aromatherapy and placing the use of *Lavender* genus as a major plant for the formulation of aromatic plant-based products. The specific harvesting method combined with an exclusive proprietary process of extraction, allows the production of a unique **Lavandin Absolute** with a characteristic scent close to the genuine lavandin flower that contains higher concentrations in β-farnesene, γ-cadinene and α-bisabolol than usual process.

Biological activities of aromatic plants extracts are linked to their chemical composition. *In-silico* artificial intelligence-based bioinformatics were performed on these molecules, providing encouraging data regarding biological activities. Nevertheless, molecular simulations are models close to reality, a concordance must be demonstrated with experimental data. Therefore, experimental studies were conducted to confirm the simulations. *In-vitro* studies showed that the **Lavandin Absolute** can increase hyaluronic acid production, cell junction markers such as Claudin 1 and Envoplakin, and epidermal differentiation markers such as Keratin 10, Keratin 14 and Involucrin. **Lavandin Absolute** is also able to reduce interleukin-6 and 8 in fibroblast cultures. This organic natural fragrance ingredient can be used to help skin hydration and skin barrier function as well as skin soothing. In addition, the application of the **Lavandin Absolute** on co-cultures of microorganisms showed a respect to skin and scalp microbiomes.

The influence of perfumes on human psycho-physiological activities are well described in the literature, and its importance is gradually increasing in the cosmetic industry. Research has made fMRI technology more accessible which allowed us to show the ability of the **Lavandin Absolute** to activate brain patterns associated with relaxation and mindfulness.

5. Conclusion

In our studies, *in-silico* bioinformatics based on artificial intelligence has shown a good predictability for the results obtained with *in-vitro* and fMRI studies. In conclusion, the **Lavender Absolute,** a natural fragrance ingredient with a characteristic odor obtained from a sustainable sourcing and unique extraction process, can bring skin benefits linked to skin barrier, skin hydration and skin soothing, combined with a remarkable ability to activate brain patterns associated with wellness.

6. References

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