IFSCC 2025 full paper (IFSCC2025-1247)

Ganoderma lucidum extract enhances skin firmness and collagen organization

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1. Introduction

Skin firmness and density declines with age, in addition to external factors related to gender, ethnic background, environment, lifestyle and weight. As with the skin of the face, the skin of the body undergoes both structural and biochemical changes with the progression of age. The stratum corneum decreases in thickness and there is a reduction in water content in different parts of the body. The most consistent change in aging skin is the morphological and compositional change in the dermal-epidermal junction, which is accompanied by a loss of key structural proteins such as collagen and elastin. However, there are also changes in the physical characteristics of dermal collagen that leads to changes in its structure, affecting the mechanical properties of the skin. These molecular changes are translated into visible signs of body skin aging including skin laxity, sagging, thinness, lines, uneven texture, and dryness. Consequently, solutions must be designed with a multi-faceted approach to address these diverse concerns. Topical application of active ingredients, such as peptides, retinoids, and certain botanical extracts, has been shown to restore firmness by remodeling the dermal matrix.

The primary objective of this study is to investigate the potential of an extract of the fungus *Ganoderma lucidum* to enhance the structure and function of the extracellular matrix (ECM), with the aim of improving skin firmness and preventing sagging.

2. Materials and Methods

2.1. Extracellular matrix proteins

To investigate the role of *Ganoderma lucidum* extract (reishi extract - RE) in skin firmness, first human dermal fibroblasts (NHDF) were incubated with varying concentrations of either the RE or *Centella asiatica* extract (CE) to assess their cytotoxicity. CE was employed as a standard ingredient given the high prevalence of this extract in firming cosmetic products. Consequently, two concentrations (0.015 and 0.031 mg/ml) were selected for the subsequent incubation of NHDF with both extracts. Following a 24-hour incubation period, the content of collagen I, elastin and hyaluronic acid was quantified by ELISA with commercial kits in the supernatants of NHDF cultures.

Collagen Type I was determined with the Human Pro-Collagen I alpha 1 DuoSet ELISA from R&D Systems (Minneapolis, MN, USA) following manufacturer's instructions. Elastin content was measured with the commercially available kit Fastin (Biocolor Ltd, County Antrim, UK) following manufacturer's instructions. And Hyaluronic acid (HA) levels were determined with the Hyaluronan DuoSet® ELISA kit from R&D Systems (Minneapolis, MN, USA). All experimental conditions were tested in triplicate.

Values were normalized to the protein content measured by the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Pittsburgh, PA, USA) following manufacturer's instructions.

2.2. Antioxidant capacity

Scavenging activity of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was performed on both extracts at varying concentrations to analyze the antioxidant capacity of RE and CE. DPPH is one of a few stable and commercially available organic nitrogen radicals and has a UV-vis absorption maximum at 517 nm. Upon reduction, the solution color fades; the reaction progress is conveniently monitored by a spectrophotometer. Because DPPH is a well-known radical and a trap ("scavenger") for other radicals, DPPH scavenging activity is a common antioxidant assay.

The DPPH assay is typically run by the following procedure: DPPH solution in methanol is mixed with the sample solution, and the reaction progress absorbance of the mixture is monitored at 517 nm for 30 min or until the absorbance is stable. In particular, we followed the Brand-Williams method as described elsewhere.

A stock solution of DPPH (Sigma, D9132-1G) was used to prepare the DPPH working solution (105 µM). Next, 190 µl of DPPH working solution was mixed with 10 µl of the appropriately diluted test samples in 96-well plates. To follow, the plate was gently shacked and incubated in darkness during 30 min at room temperature. Absorbance at 517 nm was then registered with a plate reader (SpectraMax M2, Molecular devices). All conditions were run in triplicates.

2.3. Atomic force microscope studies

The impact of RE on collagen organization and structure, as well as on the skin biomechanical properties was investigated in abdominal skin explants using an atomic force microscope (AFM). For this study, skin explants from one unique donor were used and incubated with or without RE. The concentration of the RE stock solution was 100 mg/ml in DMSO, and it was diluted in culture medium to 0.031 mg/ml to prepare the working solution. Adipose tissue was removed from the abdominoplasties before the experiments. From these skin explants, several punches (three per condition) of the same size (1.20 cm^2) were cut and placed in survival medium (Gibco DMEM + antibiotic cocktail, ThermoFischer) but not completely immerged. This ensures proper hydration and nutrition of the skin punches through dermis absorption. Skin punches were placed at controlled temperature and humidity during the experiment (relative humidity: > 93% at 37°C). The working solution was added to the culture medium for each punch at a concentration of 0.031 mg/ml for five days, twice a day. Then, skin punches were frozen in liquid nitrogen and conserved at -80°C before cryosection. Cryosections of 20 µm thickness were performed using a Cryostat (Leica CM3050S), at -21°C (one cryosection per punch).

AFM data acquisitions – Measurements of mechanical properties

The Atomic Force Microscope (AFM) used in this study is a Bioscope Resolve (Bruker) on which is added an epifluorescence microscope (Leica DMi8). This configuration allows the precise positioning of the AFM probe on the sample. This unique combination also allows us to acquire correlative images from mechanical to fluorescent acquisitions. AFM mechanical measurement is based on the atomic scale interaction between a fine tip placed on a flexible cantilever and the skin section. Consequently, the force volume in the dermis is acquired, which corresponds to a multitude of measurable points from which mechanical properties are extracted including the elastic modulus (measured in kPa).

2.4. Statistical data analysis

The efficacy data are expressed in numbered data and are submitted to a suitable statistical treatment (ANOVA, Student-t Test, Wilcoxon Signed-Ranks Test or Mann-Whitney Test) for all the comparisons within the measurement times and within the treatments.

All the calculations were performed using GraphPad Prism® v10 software. A 95% level of significance was adopted.

3. Results

3.1. Collagen I, elastin and hyaluronic acid

The results showed that there was an increase in the synthesis of collagen I and elastin for both extracts, at the two concentrations as expected (Figure 1). However, the rise was significantly higher for RE with a 55% increase in collagen I and 58% in elastin compared to a 16% and 19% increase respectively, for CE. There was no significant difference between CE and RE at the dose of 0.015 mg/ml.

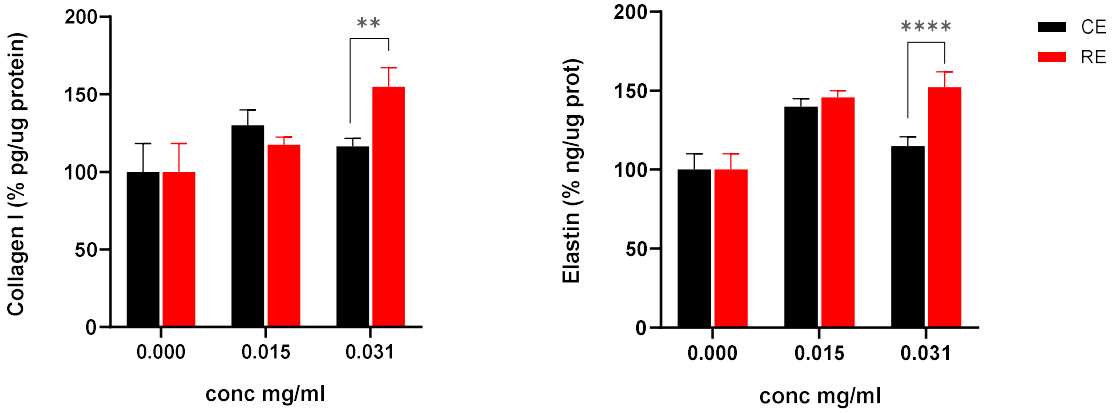

Figure 1. (a) Collagen I and (b) elastin content in NHDF. Results are expressed in percentage of pg molecule/µg protein for both extracts as compared to the control value. **p<0.01, ****p<0.0001

In terms of hyaluronic acid content (Figure 2) we also observed an increase in its synthesis with concentrations of 0.015 and 0.031 mg/ml for both extracts. As in the case with collagen I

and elastin, a marked increase in hyaluronic acid levels is observed with RE (53%) as compared to CE (14%) at the highest concentration.

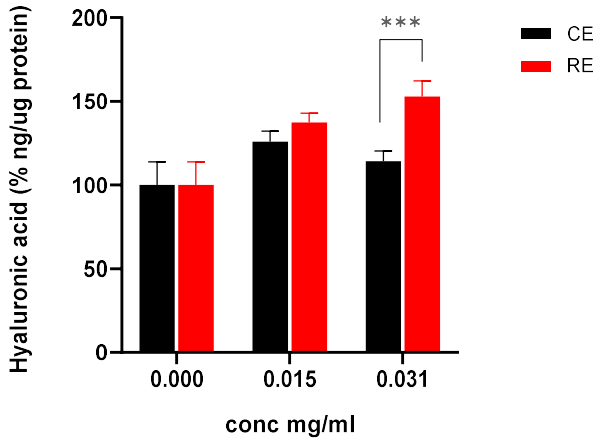

Figure 2. Hyaluronic acid synthesis in NHDF. Results are expressed in percentage of pg HA/µg protein for both extracts as compared to the control value. ***p<0.001

These results show an impact of RE in the ECM since it is increasing the synthesis of the most abundant molecules.

3.2. Antioxidant capacity

It has been described that *Ganoderma lucidum* has antioxidative properties due to its high chemical composition of phenolic compounds, polysaccharides, triterpenes and others [1]. To investigate its potential, a DPPH scavenging test was performed. As observed in Figure 3, a dose response increase in antioxidant capacity was observed for both extracts, CE and RE, from a concentration of 0.031 mg/ml up to 0.25 mg/ml. The scavenging capacity of RE exhibited a sustained increase, reaching a maximum of 77% at a concentration of 0.25 mg/ml which was statistically significant when compared to CE, which reached 64% at the same concentration.

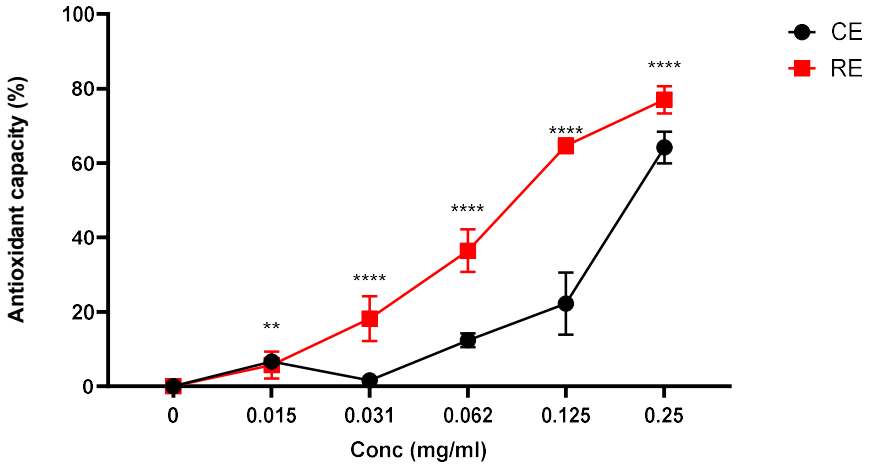

Figure 3. DPPH scavenging activity for CE and RE. Results are expressed in percentage compared to control. ** p<0.01, **** p<0.0001 vs CE

As demonstrated by this assay, RE presents a strong antioxidant effect which could help to enforce skin firmness.

3.3. Atomic force microscope (AFM)

The biomechanical capacities of the dermis are responsible for providing the skin with its elasticity and resistance to stretch. Changes in dermis mechanics occur during intrinsic aging, photo-damage, hypertrophic scarring, and fibrosis. In addition, mechanical alterations of the dermal ECM influence the activity of residing fibroblasts, altering different functions such as cell proliferation, migration and gene expression [2], [3]

Because of the increase in the synthesis of different proteins from the ECM, we decided to analyze the mechanical properties of the dermis (reticular and papillar) in the presence of RE using the AFM.

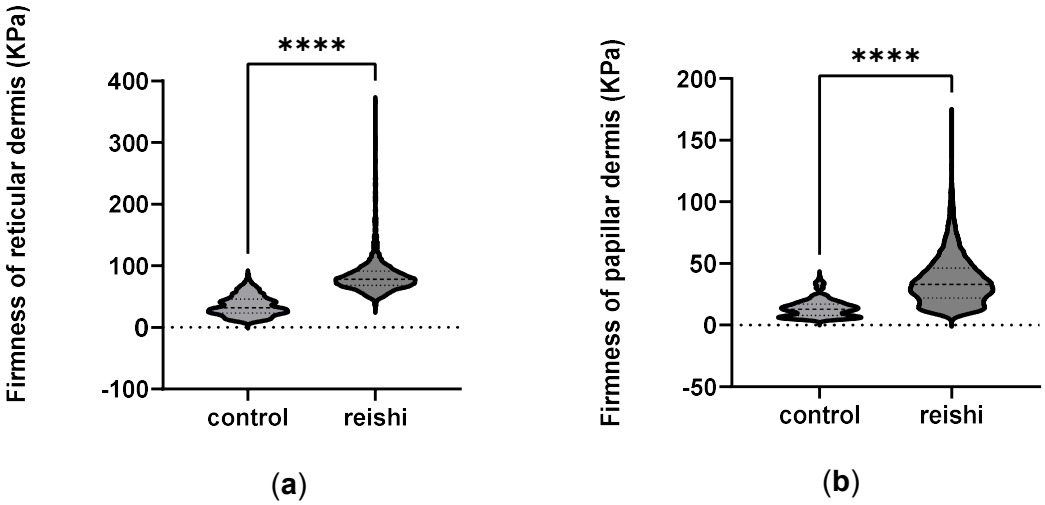

Figure 4. Elastic modulus of reticular dermis (a) and papillar dermis (b).

As observed in Figure 4, RE resulted in a highly significant increase (167%) in the firmness of the papillary dermis and a significant increase also (127%) in the reticular dermis. These results indicate that RE is able to improve body skin firmness by strengthening the dermis.

The analysis of collagen fiber orientation provides information on the capacity of the collagen network to support the skin. This analysis in the skin explants revealed that RE modifies the organization of the collagen network (Figure 5). The images of collagen fibers revealed that the control samples exhibited a uniform distribution of collagen fibers, with a range of less than 30°. Moreover, it tends to lean towards the ends of the orientation range (0 or 180°), closer to the horizontal relative to skin surface. Finally, the skin explant treated with RE displayed a more heterogeneous orientation, and it spreads around 50 to 70° of the orientation range.

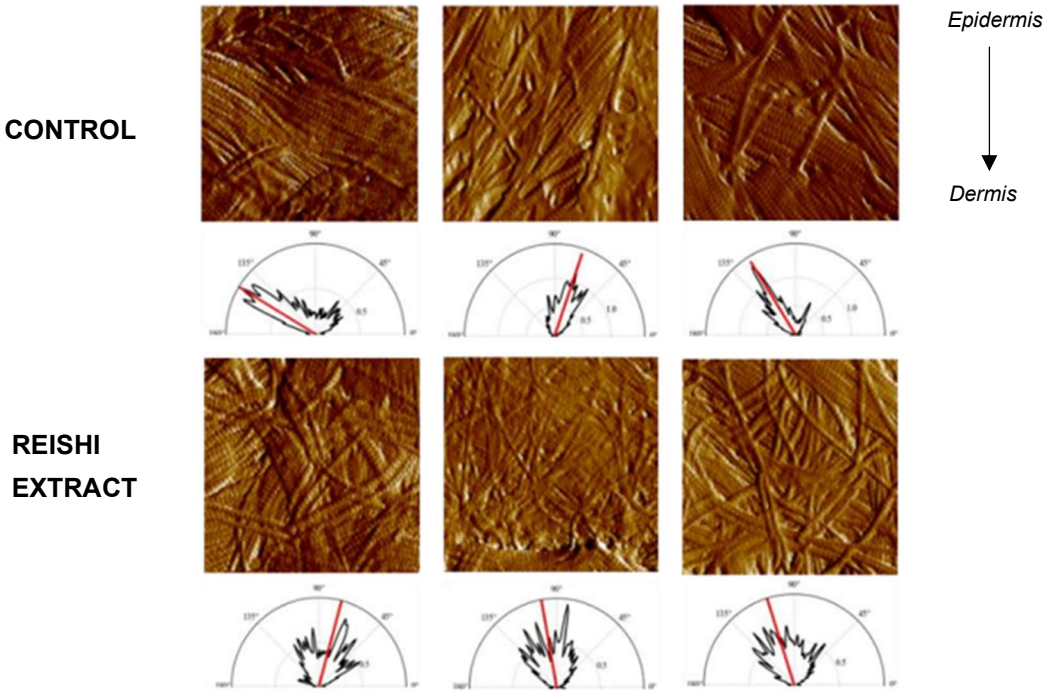

Figure 5. Collagen fiber orientation and distribution depending on the treatment.

This finding suggests that the RE may influence the collagen organization, thereby increasing dermal tension and resulting in a firming effect on the skin.

4. Discussion

Skin firmness is primarily determined by the structural integrity of the dermal extracellular matrix, particularly collagen and elastin fibers, which provide tensile strength and elasticity. With aging and environmental exposure, collagen production declines and elastin become fragmented, leading to decreased skin firmness and the appearance of sagging. Strategies to improve skin firmness often focus on stimulating fibroblast activity to enhance collagen and elastin synthesis. Our study demonstrates that RE enhances the synthesis of collagen I and elastin, as well as an increase in hyaluronic acid. Furthermore, the robust antioxidant capacity observed contributes to enhanced protection and functionality of both the fibroblasts and the overall dermis.

Aging has a well-documented impact on the mechanical and structural properties of the skin, primarily due to alterations in the ECM. The content and organization of key ECM proteins such as collagen and elastin decline with age, contributing to reduced dermal stiffness, particularly in the reticular dermis where collagen is predominantly localized [4]. This degradation is accompanied by a decrease in collagen fiber thickness and significant morphometric changes, notably in fiber orientation. In youthful skin, collagen fibers display a heterogeneous orientation, enabling mechanical support in multiple directions. In contrast, aged skin exhibits a more homogeneous, horizontally aligned fiber pattern, which diminishes support and contributes to skin laxity [5].

The structural analysis made with AFM further confirmed differences in dermal architecture due to RE. The results in our study indicate that the range of fiber orientation seems to be more comparable to younger skin, since it displayed a more heterogeneous fiber orientation

compared to control samples. These structural modifications align with the observed biomechanical enhancements, suggesting that the active ingredient may modulate collagen fiber orientation or affect fibroblast-mediated matrix tension to enhance skin firmness as a mechanism of action.

5. Conclusion

Taken together, these *in vitro* findings demonstrate that *Ganoderma lucidum* extract (RE) exerts a potent skin firming effect, attributed to its strong antioxidant activity and its capacity to enhance the synthesis of key extracellular matrix components, including collagen I, elastin, and hyaluronic acid. Biomechanical analysis further reveals an increase in dermal stiffness and a heterogeneous reorganization of collagen fibers, suggesting that RE not only promotes ECM production, but also influences collagen architecture to enhance dermal tension. These results confirm that RE is a highly bioactive compound with significant antioxidant and ECM-modulating properties, providing compelling evidence of its efficacy in improving body skin firmness.

6. References

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