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AN INSTANT RELEASE ROYAL JELLY-CYCLODEXTRIN INCLUSION COMPLEX FOR SOFT SKIN AND HAIR APPLICATIONS

APIVITA

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Background & Summary

Royal jelly (RJ) is a yellowish-white, gel-like substance produced by the hypopharyngeal and mandibular glands of young worker bees. RJ offers numerous health benefits, including antimicrobial, anti-inflammatory, anticancer, antioxidant, antidiabetic, immunomodulatory and anti-aging properties. Moreover, RJ has gained attention for its potential to combat skin aging as it is rich in key bioactive compounds such as 10-hydroxy-2-decenoic acid (10-HDA) and polyphenols that confer antioxidant and photoprotective activities on skin [1,2]. However, their extraction and use are challenging due to their stability, as they degrade when exposed to elevated temperatures, light and oxygen.

How can the stability limitations of RJ be overcome to enhance its use in cosmetic formulations?

The present study intends to eliminate the stability disadvantages of RJ, offering protection and instant release of the 10-HDA and the polyphenols that it contains aiming to enhance its bioactivity in cosmetic applications.

We performed extraction and inclusion of bioactive components from fresh, untreated RJ into a β-hydroxylpropyl cyclodextrin (HPβCD) system, composed solely of natural and safe ingredients, which results in the instant release (IR) of the incorporated polyphenols and 10-HDA.

This RJ-IR formulation was further incorporated in cosmetic formulas to evaluate the benefits of an instant activity.

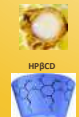
We show that:

- **RJ-IR modulates the expression of stress factors *in vitro* in hair follicle dermal papilla cells (HFDPCs)**
- **RJ-IR incorporation in shampoo and hair conditioner reduces hair porosity of hair shaft, upon urban pollution stress or baseline conditions and restores hair integrity**
- **RJ-IR incorporation in a face cream effectively repairs skin barrier function**

Materials & Methods

RJ-IR preparation

RJ (≥1.6% 10-HDA) was dissolved to 5.5% w/w in deionized water and 1,3-propanediol under stirring. HPβCD (20% w/w) is pre-dissolved in water and mixed in. The mixture was sealed and stored at 5–7 °C for 8 h, then cold-filtered (5–0.45 µm). pH is adjusted (3.5–7.5) if needed. Subsequently, total polyphenols and 10-HDA are then quantified.



Gene expression analysis

Total RNA (500 ng) was extracted and reverse-transcribed using standard kits. Gene expression was analyzed by qPCR using the ΔΔCt method, with GAPDH as the reference gene.

In Vivo and Ex Vivo Application Studies

Hair Care Application:

0.5% w/w in shampoo and hair care mask as a routine treatment:

- applied on hair shafts. The shafts were further exposed to urban dust and UV-A, stained with a fluorophore, cryosectioned (5 µm), and imaged using an epifluorescence microscope (Evos M5000). Images were analyzed with ImageJ
- used by 5 volunteers. Fibers were collected and observed using scanning electron microscopy (SEM)

Skin Application:

A 0.5% w/w RJ-IR face cream was applied by 10 volunteers, and transepidermal water loss (TEWL) was measured using a Tewameter® TM HEX.

Results &Discussion

- **Upregulation of Nrf2 gene expression in HFDPCs was observed after 24-hour exposure to RJ-IR, indicating activation of cellular antioxidant pathways**

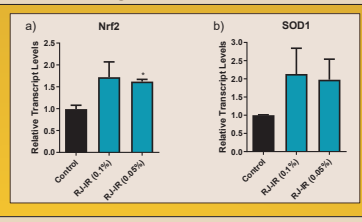


Figure 1. Gene expression of Nrf2 (a) and SOD1 (b) after exposure of HFDPCs to RJ-IR 0.05% v/v. Non-parametric t-test, comparison with the control. * p-value <0.05"

- **The RJ-IR routine (shampoo + hair conditioner) showed significant repair efficacy against urban pollution induced alterations on hair fiber integrity and porosity *ex vivo***

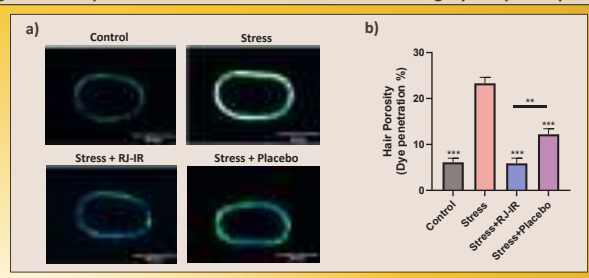


Figure 2: Hair integrity and porosity. a) in situ visualization of fluorescein diffusion on cross-section of hair. The fluorescence emission signal for fluorescein was obtained by using specific excitation and emission wavelengths (498/517 nm). Fluorescein is visualized in a color range (low levels in dark, high levels in bright colors). b) The quantification of fluorescein diffusion on hair is reported for each experimental group and expressed as % values of fluorophore penetration in the hair shaft and shown as mean ± S.D. ***, p < 0,001; **, p < 0.01, one-way ANOVA and Dunnett's post-hoc test for multi-comparisons analyses vs stress group or Unpaired t test with Welch's correction for binary comparison.

- **The RJ-IR routine (shampoo + hair conditioner) improves hair uniformity, smooths the surface, and restores cuticle integrity *in vivo***

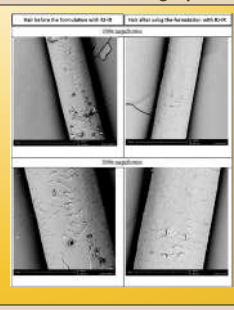


Figure 3: Imaging of hair strand samples isolated from volunteers using scanning electron microscopy, before (left) and after (right) the use of shampoo enriched with 0.5% w/w RJ-IR and the use of a hair cream enriched with 0.5% w/w RJ-IR. Magnification 1000X and 2000X.

- Cream with 0.5% RJ-IR leaves a protective layer on the skin which contributes to the reduction of water evaporation from the horny layer of the epidermis

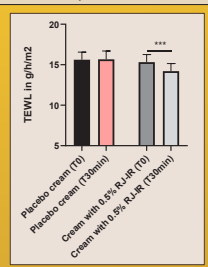


Figure 4: TEWL measurements before (T0) and 30 minutes (T30) after application of cream with 0.5% RJ-IR or placebo. A reduction in TEWL was observed 30 minutes after application of the RJ-IR cream. Data are mean ± SEM; n = 10 per group. p < 0.001, paired t-test.

References

[1] Chen, C., & Chen, S. Y. (1995). Changes in protein components and storage stability of Royal Jelly under various conditions. *Food Chemistry*, *54*(2), 195–200

[2] Rizki, P. A., Krisnamurti, D. G. B., Reksodiputro, M. H., Farida, S., Koento, T., Rachmadi, L., Wuyung, P. E., Wibowo, H., & Boesoirie, S. F. (2023). STABILITY EVALUATION OF ROYAL JELLY GEL. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *71*(2), 89–96

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An instant release royal jelly-cyclodextrin inclusion complex for skin and hair applications

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