'WHERE BEAUTY, SCIENCE AND INNOVATION MEET!'
32nd IFSCC Congress
London 19–22 September 2022

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Standardized phytocomplex of *Perilla frutescens L.* derived from in vitro cell cultures: maintenance of skin integrity and use in vaginal gel formulations

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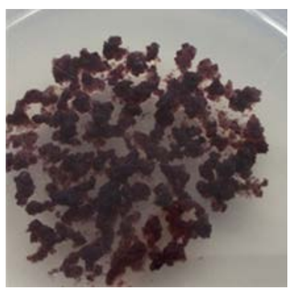
Introduction:

Plant cell culture technology is a technique for growing of plant cells under strictly controlled environmental conditions that makes it possible to provide preparations with a **standardized content** of active substances and with a high **safety profile** for the consumer. *Perilla frutescens* L., also known as Shiso, is a specie of Perilla that belongs to the Lamiaceae family, commonly used as an aromatic and medical plant[1]. *Perilla frutescens* phyocomplex (PFP), derived from *in vitro* plant cell cultures, has a high and standardized content of **rosmarinic acid** (RA) **and anthocyanins**[2].

The object of this study is to demonstrate the activity of this phytocomplex to maintain vaginal mucosa integrity acting with anti-inflammatory and hydrating activity and its application in intimate gel formulations.

Materials & Methods:

Collected plant and confirmed origin by fingerprint analysis



Explant and selected cell line in solid and liquid medium

Inoculation in bioreactor to obtain phytocomplex

Phytochemical analysis: UPLC-DAD

Biological test: TNF-α, IL-1β, IL-6 dosage Tight junction and skin barrier protein evaluation

Phase	Ingredients	Composition (%)
A	Water	Add until reach 100
	Glycerin	3 - 0
	Tamarindus Indica Seed Polysaccharide	0.5
	Rheology Modifier	Varies
	PFP glycerin suspension	0 - 3
B	Phenoxyethanol, Ethyhexylglycerin	0.9
C	Buffering Agent	Add until reaching pH 5-5.5

Cosmetic formulations with PFP, Rheological analysis and Stability test

Conclusions:

Perilla frutescens* phytocomplex** produced by *in vitro* plant cell culture technology, with a high and standardized association of rosmarinic acid and anthocyanins showed in vitro an anti-inflammatory activity and the ability to repair skin barrier functions. ***Succinoglycan gum and ***Sclerotium gum*** have proved to be the most suitable natural polymers for their contribution in the elastic component of the gel, since the weak-gel structure can be the optimal one **to keep the active in suspension**.

Ternary associations between these **natural polymers** seem to be the most suitable for obtaining products with the required characteristics of texture and stability and to better maintain PFP in suspension. PFP can be used as a **new cosmetic ingredient for intimate products** with **sustainable and safety** features related to the production process.

Results & Discussion:

Perilla frutescens phytocomplex produced by in vitro plant cell culture technology, is characterized by a **high and standardized content of RA and anthocyanins**. The content of total polyphenols identifies by their characteristic spectrum with λmax at 330 nm and expressed as equivalent of RA was 2.35 ± 0.16% w/w. The content of total anthocyanins identifies by their characteristic spectrum with λmax at 520 nm and expressed as equivalent of cyanidine-3-O-glucoside was 0.10 ± 0.02% w/w.

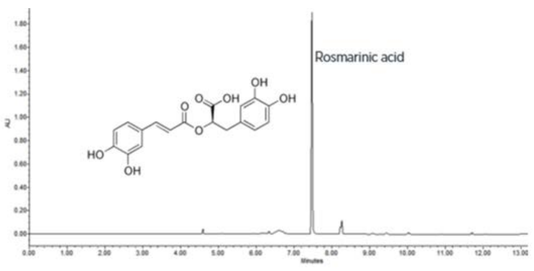


Figure 1. Chromatographic profile of the PFP extract at 330 nm wavelenght. The main peak at retention time 7.5 min corresponds to RA.

PFP has been confirmed as a product with a **high anti-inflammatory capacity** and the over-release of TNF-α, IL-1β and IL-6 was almost completely inhibited by the treatment. Treatment with PFP was able to prevent damage induced by LPS+H_2O_2 by preserving the **tight junctions** ZO-1 and occludin expression in keratinocytes.

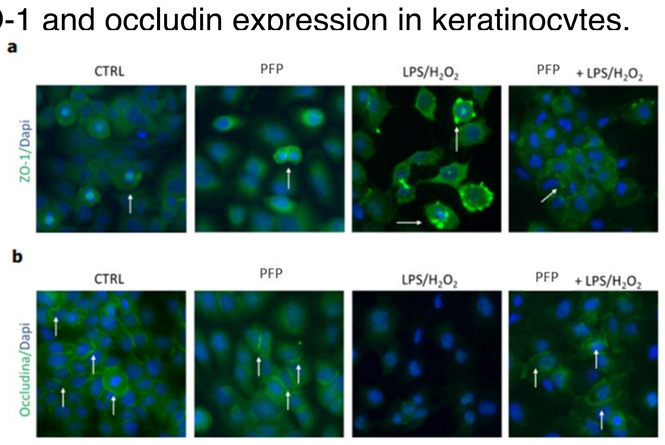


Figure 2. Immunofluorescence staining analysis revealed the compromise of ZO-1 and occluding junction expression in the keratinocytes following stimulus with LPS + H_2O_2 for 3 hours compared to the control.

Rheological analyses show that in gels with weak-gel characteristics the contribution given by PFP is hardly perceptible. Moduli values between 10 and 1000 Pa, typical of a **weak-gel structure**, can be optimal to keep the active in suspension. *Succinoglycan gum* (R) and *Sclerotium gum* (A) have proved to be the most suitable **natural polymers** for the formulation of gels with this type of active ingredient, giving a contribution to the elastic component of the system by increasing its stability.

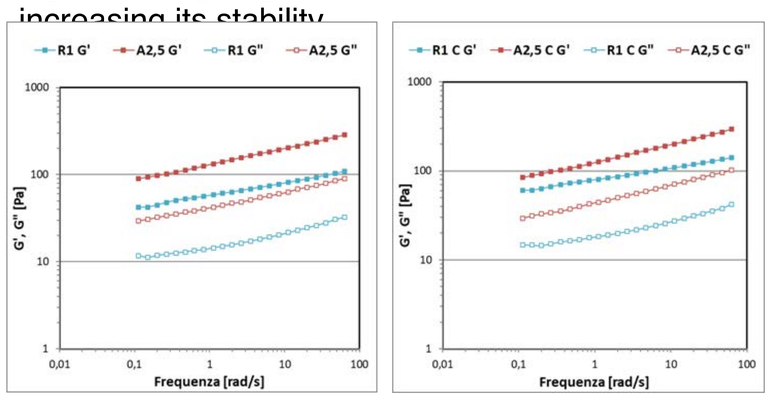
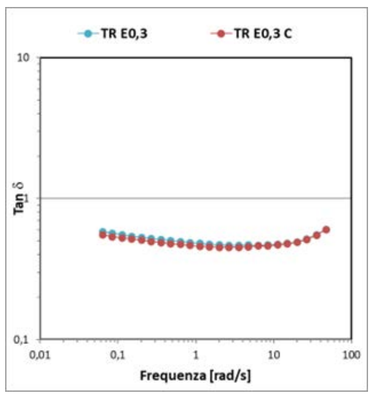


Figure 3. Frequency sweep analysis of gel formulations with *Succinoglycan gum* (R) and *Sclerotium gum* (A) without and with PFP glycerin suspension (C)

Ternary associations of polymers, between Tamarind Seed Polysaccharides, Succinoglycan gum and Esaflor HM22, in which there is a correct balance between elastic and viscous modulus, seems to be the one with the best structural properties in which the active ingredient does not significantly change the characteristics of the structure but is stable in the formulation.

Figure 4. Ternary systems with the association of Tamarind Seed Polysaccharides (T) and Succinoglycan gum (R) with and without the active ingredient.



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1. Ahmed HM (2018) Ethnomedicinal, Phytochemical and Pharmacological Investigations of Perilla frutescens (L.) Britt. Molecules 24:1.
2. Pressi G, Bertaiola O, Guzzo F, Biagi M. Phytocomplex and extract of a meristematic cell line selected of Perilla frutescens. Patent ITA102020000028230/ PCT/ IB2021/057560. 24 November 2020.