Beyond retinoids: a natural cyclic peptide ingredient for safe and efficient dual action against skin aging

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Abstract

As skin ages, it becomes thinner, less elastic, and more wrinkled. Retinol is the gold standard anti-aging skin care ingredient but can cause irritation. Bakuchiol is a natural alternative that targets similar pathways. Still looking for natural molecules with potent activities even at low concentration, peptides have recently surged in popularity. First mainly linear, cyclic peptides gain in interest boasting superior skin penetration, stability, and performance. For the first time, this study evaluated a novel natural extract from Prince Ginseng (PHE) enriched in cyclic peptides for anti-aging effects compared to retinol and bakuchiol. Transcriptomic analyses showed PHE stimulates genes involved in retinoid metabolism, epidermal barrier function, and dermal extracellular matrix production. PHE boosts collagen I synthesis in fibroblasts comparably to retinol. Using 3D dermal spheroid model, PHE increases SPARC and collagen I expression, suggesting improved matrix organization, exceeding retinol's effects. Clinically, PHE improves skin firmness, brightness, facial contour, and sagging. Unlike retinol, PHE is a gentle yet highly effective plant-based solution combining retinoid metabolism modulation with strong

anti-aging activity. It enhances epidermal barrier function and dermal matrix quality for a more structured, youthful appearance. PHE presents an ideal natural cyclic peptide-enriched alternative to retinol with superior anti-aging performance.

Keywords: cyclic peptides; anti-aging; collagen; dermal matrix; retinol alternative

Introduction

Skin aging is driven by intrinsic (mainly genetic factors) and extrinsic factors (such as UV exposure, pollution,…). The global skin aging process involves remodeling and deterioration of epidermal, dermal, and junctional elements [1]. This convergence of features ultimately manifests as fragility, dryness, wrinkles, and pigmentation changes over time [2].

Retinoids like retinol and retinaldehyde are famous anti-aging skin care ingredients, regulating cellular growth, proliferation, and differentiation as well as cell metabolism. In the body, retinoïds, provided by nutrition as precursors, are metabolized into all-trans retinoic acid (atRA). This active form binds to nuclear receptors in the cells, called RARs and RXRs, which act as ligand-activated transcription factors that regulate gene expression by interacting with retinoid response elements (RAREs) on target genes. In skin cells, this leads to increased collagen production, faster cell turnover, improved barrier function and thickness, MMPs inhibition, and decreased pigmentation [3].

Several steps define the retinol metabolism. Briefly, retinol coming from circulation forms a complex with a retinol binding protein (RBP4) that interacts with cell membrane protein to release retinol inside the cell then transforming it into retinoic acid active form through a series of enzymatic reactions. This process involves retinol dehydrogenases (RDHs) such as Retinaldehyde Reductase 9 (DHRS9) or Xanthine dehydrogenase (XDH), first oxidizing retinol

into retinaldehyde. This first step of the atRA formation is reversible. Retinaldehyde dehydrogenases (RALDHs), such as ALDH2 and ALDH9A1, then oxidize retinaldehyde into atRA. This second step is irreversible. The cytochrome P450 enzymes such as CYP1A family, especially CYP1A1 and CYP1A2, also play major roles in hydroxylation and oxidation of retinol and retinaldehyde [4]. While CYP1As have a role in forming atRA [5], CYP26s break down atRA as part of catabolism. Intracellular carrier proteins regulate retinoid availability and actions. Beyond RBP4 transporting retinol in circulation, CRBP facilitates retinol uptake and conversion to active metabolites or storage. CRABP2 shuttles RA to the nucleus to bind RARs while CRABP1 sends RA to CYP catabolism.

atRA act then by activating nuclear retinoic acid receptors (RARs) - RARα, RARβ, and RARγ - which have different affinities, expression, and gene targets. In skin, RARs form heterodimers mainly with retinoid X receptors (RXRs). RARγ makes up ~90% of skin RARs and RXRα ~90% of RXRs. So skin is regulated primarily by RARγ-RXRα heterodimers that bind retinoic acid response elements in DNA, triggering transcription of retinoid-responsive genes. While RAR are specific to atRA, RXRα also partners with other receptors [6].

Retinol, while effective and well-recognized for skin anti-aging benefits, is also known to cause side effects like irritation and peeling. Encapsulating retinol in delivery vehicles like liposomes is a way to reduce these drawbacks. The development of topical retinoids selectively targeting the RARγ receptor subtype is also a strategy to mitigate these issues, improving efficacy and safety over conventional retinoids [7] [8].

An alternative approach has been to discover natural compounds that can replace retinol. One such compound that has gained attention is bakuchiol, derived from the Babchi plant. Bakuchiol targets similar cellular pathways as retinol to stimulate collagen production, reducing wrinkles and improving skin firmness and pigmentation. Unlike retinol however, bakuchiol does not bind to retinoic acid receptors [9]. Despite showing promise as a natural alternative, recent studies

have reported allergic reactions and contact dermatitis to bakuchiol [10] [11], highlighting the need to explore other well-tolerated sources that can mimic the skin anti-aging effects of retinol without drawbacks.

In this way, we propose here a specific extract of *Pseudostellaria heterophylla* root (PHE), enriched in a specific cyclic peptide, heterophyllin B, as a perfect natural and mild alternative to retinol. *Pseudostellaria heterophylla* is an adaptogen used in Traditional Chinese Medicine to enrich qi, nourish yin, and generate fluids in cases of deficiency [12] [13]. Modern research has confirmed some of the plant's pharmacological activities, including immunostimulant, anti-inflammatory, antioxidant and liver cell-protective effects [14]. Heterophyllin B is a cyclic octapeptide, abundant in *Pseudostellaria heterophylla* tuberous roots. Cyclic peptides are bioactive plant molecules involved in their defense against pathogens. Their closed cyclic structures enhance stability and resistance to degradative enzymes compared to linear peptides [15] [16].

We studied the ability of this cyclopeptide enriched extract to replace retinol by demonstrating that PHE both optimizes retinol metabolism in the skin and provides its own anti-aging signature with wide epidermis and dermis benefits. Moreover, in two independent clinical studies we compared PHE to bakuchiol or retinol, showing an optimal effect of PHE on anti-aging signs such as wrinkles reduction and anti-sagging effect, either equivalent or better than the reference molecules.

Materials and Methods

1. Plant extract studied

PHE production process has been specially designed from the plant species *Pseudostellaria heterophylla*, with a fractionation step to enrich the root extract in heterophyllin B (and 7 other cyclopeptides). Only natural-based solvents are used during the process.

2. Skin explants culture

Skin explants were produced from abdominal surgery of a female donor (age 34 years old, caucasian, phototype III). PHE, formulated at 0.3%, was applied topically (5 mg/cm²) as well as placebo formulation (Water, Dicaprylyl Ether, Ammonium Acryloyldimethyltaurate/VP Crosspolymer, Dicaprylyl Ether, Citric Acid, Sodium Citrate, Phenoxyethanol, Methylparaben, Ethylparaben, Fragrance) for 24 hours in a humidified incubator at 37°C and 5% CO2. All experimental conditions were performed in n=3.

3. Cell culture

Primary normal human keratinocytes (NHEK) at passage 3, coming from a donor aged of 47 years old, were treated 24 hours after seeding in 6 wells plate by PHE at 0.15% for further transcriptomic analysis.

Primary normal human dermal fibroblasts (NHDF) at passage 6, coming from a donor aged of 47 years old, were treated 24 hours after seeding in 6 wells plate by PHE at 0.01% for further transcriptomic analysis.

Primary normal human dermal fibroblasts (NHDF), coming from donors aged of 35 years and 50 years old, were used to study the pro-collagen I synthesis. The cells were exposed 72 hours to

PHE at 0.1% or heterophyllin B at 0.0001% (dose equivalent to the one contained in PHE 0.1%) or Retinol at 0.001% or bakuchiol at 0.001%. TGF-β1 at 10 ng/mL was used as a positive control.

4. Transcriptomic analysis

4.1 Skin explants

At the end of the topical treatment, samples were mechanically homogenized using a Precellys® Evolution homogenizer (Bertin Instrument) and total RNA was extracted from each sample using TriPure Isolation Reagent® (#11667157001, Roche, Basel, Switzerland) according to the supplier's instructions. The amount and quality of RNA were evaluated using capillary electrophoresis (Bioanalyzer 2100, Agilent technologies). The complementary DNA (cDNA) was synthetized by reverse transcription of total RNA or mRNA in presence of oligo(dT) and «Transcriptor Reverse Transcriptase» (#3531295001, Roche Life Sciences, Basel, Switzerland). The cDNA quantities were then adjusted before PCR step. PCR were performed using the « LightCycler® » system (Roche Molecular System Inc., Basel, Switzerland) according to the supplier's instructions.

The relative expression value was calculated with the formula: $(1/2^{\text{number of cycles}}) \times 10^6$. The PCR array used included 3 reference genes (GAPDH, RPS28 and ACTB). These housekeeping genes were used for data normalization since their expression is constitutive and theoretically stable. Consequently, the level of expression of the target markers was compared to the mean expression level of these 3 markers for all test conditions.

4.2 Cell culture

After treatment, cells were harvested, and mRNA extraction was performed thanks to RNeasy Plus Mini Kit (#74134, Qiagen, Venlo, Netherlands). mRNA quality evaluation was performed using a fragment analyzer (Agilent technologies, Santa Clara, CA, USA) and a High Sensitivity RNA Analysis Kit (#DNF472-0500, Agilent technologies). Reverse transcription step allowing the obtention of cDNA template was performed on 0.5µg of RNA with the CFX96 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). Then, cDNA templates were diluted with nuclease free H_2O and mixed either with SsoAdvanced Universal SYBR Green Supermix (#1725270, Bio-Rad Laboratories, Hercules, CA) for retinol metabolism study or with TaqMan Fast Advanced Master Mix (#4444556 Life technology Waltham, MA, USA) for anti-aging signature study. 20µL of diluted cDNA were added on each well of predesigned 96-well panel for retinoid metabolism evaluation (retinol metabolism H96, Bio-Rad Laboratories, Hercules, CA, USA) or customized TaqMan Arrays (home designed 96 genes plates focused on dermal or epidermal compartment) (Life technology Waltham, MA, USA). PCR was run on a CFX96 thermal cycler. Raw data and statistic (Student's t-test) were analyzed on the software Bio-Rad CFX manager (for anti-aging signature study) or with a home developed tool (for retinol metabolism). PCR arrays included 3 reference genes (GAPDH, 18S and HPRT1) used for data normalization.

4.3. Statistical analysis

All the data were expressed in percentage of increase in comparison to the placebo condition. Statistical comparisons were performed by an unpaired Student’s t-test, considering a p-value < 0.05 as significant versus control group.

5. Pro-collagen I quantification

After treatment, cell culture supernatants were collected. Type I pro-collagen was measured by ELISA according to the instructions of manufacturer (#DPCA00, Bio-techne, Minneapolis, MN, USA) and compared to placebo. Total amount of proteins in cells was measured for each condition by a Bradford assay for pro-collagen I content normalization (pg of pro-collagen I/µg proteins). All values were expressed as mean +/- standard deviation (SD). Results for both fibroblasts (35 and 50 years old) were combined. Statistical analysis was performed using Dunnett test and a p-value < 0.05 was considered as significant compared to control condition or PHE.

6. Scaffold-free dermal spheroid model

Briefly, human dermal fibroblasts from a 50-year-old female donor were amplified for 7 passages, then put 3 days in Akura® plate (InSphero, Zürich, Switzerland) to begin the cell aggregation. Aggregates are then transferred in Akura Plate V2 (TRAP) for spheroid formation for 3 days. After this period, treatments with retinol at 0.0001% or PHE at 0.01% started. Samples were collected at D7 or D14 and fixed in formalin buffered solution 10% for histological analysis. After fixation, samples were incubated with anti-SPARC (#ab225716, rabbit polyclonal, Abcam, Cambridge, UK), anti-Pro-collagen I (#abx132015, mouse monoclonal, CliniSciences, Nanterre, France), and anti-Fibrillin I (#LS-B5512, mouse monoclonal, LaboSpace, Milano, Italy) primary antibodies overnight at 4 ◦C. Alexa 555 donkey anti-rabbit (A32724, Life Technologies, Waltham, MA, USA) and Alexa 488 goat anti-mouse (A32723, Life Technologies, Waltham, MA, USA) were used as secondary antibodies while nuclei were stained with DAPI (F6057, Merck, Darmstadt, Germany).

The histological samples have been analyzed (20X magnification) with Microscope THUNDER imager 3D (Leica Microsystems, Wetzlar, Germany) acquired with K5 (fluorescence) camera

and processed with LASX 3.7.5 software. 3D whole spheroids were observed on microscopy confocal dishes directly, 6-10 microtissues were acquired for each condition at least. For each sample a Z-stack was performed to observe the signal distribution in the whole volume of spheroids. To reduce the fluorescence noise, all acquisitions are elaborated with High Content Imaging. Marked area were normalized to the number of nuclei for each spheroid. Statistical analysis was performed using t-test and a p-value < 0.05 was considered as significant versus control condition.

7. Clinical evaluations for anti-aging benefits

The recommendations of the Declaration of Helsinki and the guidelines of the International Conference on Harmonization Good Clinical Practice were observed for both clinical studies

7.1. Clinical study on wrinkles and dermis density compared to retinol

To demonstrate clinically the potential of PHE as a natural alternative to retinol, we conducted a clinical study on 20 Caucasian women between 45 and 65 of age, presenting moderate dry skin (corneometry between 30 and 45 A.U.) and moderate to very severe signs of aging (grades 2-4 Eiben-Nielson photonumeric scale for wrinkles and loss of elasticity). They were asked to apply formulations containing PHE at 1% or retinol at 0.3% twice a day in randomized half-face (Water, Ammonium Acryloyldimethyltaurate/VP Crosspolymer, Dicaprylyl Ether, Citric Acid (and) Sodium Citrate, Phenoxyethanol (and) Methylparaben (and) Ethylparaben, Fragrance, Retinol (and) Polysorbate 20 (and) BHA (and) BHT +/- *Pseudostellaria heterophylla* extract (and) Glycerin (and) Water).

7 volunteers were removed from the study due to undesirable effect that prevents continuing with the application protocol. These effects were observed on the side of the face with the use of retinol formulation. Volunteers reported skin redness with peeling and dryness.

The study lasted 28 days, with an intermediate timepoint at D14. The 3D skin topography parameters were recorded from full face 3D reconstructions, using AEVA-HE (Eotech, Plymouth, MI, USA) at day 0, 14 and 28. Dermis density was measured in the cheek using an Ultrascan UC22 Echograph (Courage + Khazaka Electronic GmbH, Koln, Germany) at day 0 and day 28. For wrinkles measurement, the volunteers were installed on the VisioTOP-500 bench (Eotech, Plymouth, MI, USA) with AEVA-HE system. 3D acquisitions of full face were done to measure wrinkles at each timepoint. For analysis, total wrinkles were detected and classified according to 3 levels: low (class 1), medium (class 2) and high (class 3) depth. A density of deep wrinkles (high, class 3) was calculated according to the following formula:

Deep wrinkles density = deep wrinkles area / total hemi-face area x 100.

Dermal density was measured using Ultrascan UC22 Echograph. Briefly, the reflected ultrasound waves are transformed into electrical impulses and result in an image shown in 256 different colours, providing automatic measurement between set lines of dermis density (ultrasound density in % and as a color value between 0 and 256 a.u.). The % variation were calculated according to the following formula:

% variation = (Dx – D0) / D0 * 100.

7.2. Clinical study on firmness, luminosity, and sagging, compared to bakuchiol

We conducted a clinical study on 34 Caucasian women, aged 45-65 years old, presenting wrinkles/ fine lines on crow's feet, a lack of firmness on face and a dull complexion. They were randomly dispatched in the 2 groups. In both groups, PHE 0.3%/Placebo (22 volunteers) and PHE 0.3%/bakuchiol 0.5% (12 volunteers), products were used twice a day in randomized half-face (Water, Ammonium, Acryloyldimethyltaurate/VP Crosspolymer, Dicaprylyl Ether, Citric Acid (and) Sodium Citrate, Phenoxyethanol (and) Methylparaben (and) Ethylparaben, Fragrance, Bakuchiol or *Pseudostellaria heterophylla* extract (and) Glycerin (and) Water).

The study lasted 56 days, with an intermediate timepoint at D28. Brightness on cheek and sagging were evaluated after 28 days of use, whereas cutometry (R0 parameter) on temples and pictures, taken with VISIA® Skin Analysis System (Canfield Scientific, Parsippany, NJ, USA), were performed at day 28 and 56. As R0 decrease represents an increase of firmness, the absolute value of R0 was used to calculate the % variation of firmness over time.

Brightness parameter L* was studied after 28 days of cream application with a Spectrophotometer® CM700-d (Konica Minolta, Tokyo, Japan) equipped with an 8 mm diameter head.

The analysis of sagging was done after D28 visit via scoring photographs by 3 trained technician experts, based on a specific scoring scale. The analysis is performed comparing the pictures taken on D0 and after 28 days of products use on hemi-face.

Results are expressed as % variation vs Day 0 for each product according to the following formula:

$$\% \text{ variation} = (Dx - D0) / D0 * 100$$

To compare the effects of products between them, we used the following formula:

$$\text{Comparison factor} = \% \text{ variation product 1} / \% \text{ variation product 2}$$

Where Product 1: formula with PHE - Product 2: placebo formula or bakuchiol formula

7.3. Statistical analysis

In these 2 clinical studies, as the tested products (chassis formulation alone or cosmetic active ingredient) were applied on half-faces of the subjects, samples are paired. To compare the two

groups (PHE vs placebo and PHE vs bakuchiol and PHE vs retinol), as well as the evolution over time (vs D0), data were analyzed with the paired Student's t-test (if the normality of the distributions is confirmed) or Wilcoxon tests (if the normality of the distributions is rejected), considering a p value < 0.05 as statistically significant. The normality of the distributions was checked using the Shapiro-Wilk test with a level of significance set at 1%.

Results

1. Improvement of retinol metabolism by PHE

First, table 1 shows that formulated PHE at 0.3% stimulates *in situ* the expression of CYP1A1, CYP1A2 and RARγ by +94%**, +109%** and +22%*, respectively, after 24h of treatment.

Combined with this result, we observed on NHEK treated by PHE at 0.15% that the active significantly improves the transcription of essential dehydrogenases (ALDHs and XDH) that convert either retinol to retinal, or retinal to retinoic acid, or both steps (table 2). The result is an increase of the intracellular pool of retinoic acid (atRA). Moreover, we observed an increase of the DHRS3, an important retinaldehyde reductase for the control of intracellular retinoic acid content.

Genes regulated by PHE at 0.3% on skin	Relative gene expression ratio between the placebo group and the treated group	
	% of increase vs placebo (0%)	p-value
CYP1A1	+94% **	0.007
CYP1A2	+109% **	0.003
RAR gamma	+22% *	0.022

Table 1: transcriptomic regulation of retinoid signalling pathway related genes. Statistics: *p<0.05 ; **p<0.01

Genes regulated by PHE 0.15% in NHEK	Relative gene expression ratio between the control group and the treated group	
	% of expression vs control (0%)	p-value
XDH	+ 128% #	0.062
ALDH2 **ALDH9A1**	+ 29% # + 19% *	0.056 0.014
DHRS3	+ 266% #	0.086

Table 2: transcriptional effect of PHE on retinoid metabolism key players. Statistics: # p<0.1; *p<0.05

Altogether, these transcriptional data on retinoid metabolism showed the capacity of PHE to improve the global retinoid homeostasis.

2. PHE has its own anti-aging signature

We demonstrated that PHE can strongly improve epidermal barrier function, hydration, and basal membrane composition by increasing expression of epidermal component such as desmoglein (+108%), filaggrin (+155%) or integrin β4 (+37%) (Table 3).

	PHE 0.15%	
Genes	% vs control (0%)	p-value
Barrier function		
ABCA12	+50% *	0.036
DSG-1	+108% *	0.018
TGM1	+56% *	0.032
CLDN-1	+36% *	0.048
Hydration		
CASP14	+185% *	0.033
FLG	+155% *	0.001
Basement membrane composition		
HSPG2	+85% *	0.011
ITGB4	+37% *	0.044
FBN1	+60% #	0.079

Table 3: transcriptional effect of PHE 0.15% on NHEK. Percentage of regulation and p-value were calculated compared to control condition. Statistics: # p<0.1; * p<0.05; ** p<0.01; ***p<0.001

Additionally, we highlighted that PHE strongly modulates genes involved in dermal matrix synthesis and organization, as well as in fibroblast stimulation by essential growth factor expression. For example, PHE improves SPARC (Secreted protein acidic and cysteine rich) expression by almost 50%*, a matricellular protein that plays an important role in regulating collagen assembly and organization [17] [18]. We observed a similar effect for FBN1 expression, a major component of elastic fibers that also plays a key role in tissue homeostasis through specific interactions with growth factors, with an upregulation by 65%*** (table 4).

	PHE 0.01%	
Genes	% vs control (0%)	p-value
Collagen network		
COL1A1	+32% #	0.072
COL3A1	+76% **	0.002
COL5A1	+41% **	0.002
COL6A1	+27% *	0.033
SPARC	+49% *	0.018
FN1	+64% **	0.002
Elastin network		
ELN	+96% **	0.007
FBLN5	+37% **	0.006
FBN1	+65% ***	0.001
FBN2	+55% *	0.011
Fibroblasts stimulation		
FGF2	+65% **	0.006
HBEGF	+67% *	0.027
IGF1R	+52% *	0.032
IGFBP3	+91% *	0.037
TGFA	+118% *	0.035
TGFB1	+45% *	0.024

Table 4: transcriptional effect of PHE 0.01% on NHDF. Percentage of regulation and p-value were calculated compared to control condition. Statistics: # p<0.1; * p<0.05; ** p<0.01; *** p<0.001

3. Stimulation of the collagen synthesis by dermal fibroblasts with PHE

As expected, both TGF-β1 and retinol are able to induce an increase in the pro-collagen I synthesis by dermal fibroblasts (+74%* and +158%*** compared to untreated condition, respectively) (Table 5). More surprisingly, bakuchiol, the famous retinol natural alternative, in our experimental conditions, induces a slight and non-significant increase in pro-collagen I.

PHE significantly boosts pro-collagen I level by +133%***, and its biomarker heterophyllin B by +95%**. This data highlights that PHE efficiency is supported by its unique content in cyclopeptides.

Condition	**Pro-collagen I content (mean pg of pro-col I/ µg of protein) +/- SD**	**% of effect vs control**	**p-value vs Control**	**p-value vs PHE**
Control	2.75 +/- 0.32	100		
TGF-β1	4.78 * +/- 0.58	174	0.01	* 0.02
Retinol (0.001%)	7.10 *** +/- 0.70	258	0.0001	ns 0.74
Bakuchiol (0.001%)	3.96 ns +/- 0.43	144	0.27	** 0.002
PHE (0.1%)	6.39 *** +/- 0.48	233	0.0001	1
Heterophyllin B (0.00001%)	5.37 ** +/-0.33	195	0.001	ns 0.4

Table 5: pro-collagen I synthesis following retinol or bakuchiol or PHE or heterophyllin B treatment versus untreated condition. Statistics: * p<0.05; ** p<0.01; *** p<0.001; ns: non-significant

4. Extracellular matrix improvement in scaffold-free dermal spheroid model with PHE

In this spheroid model, we observed that retinol did not modulate SPARC expression after 7 days of treatment (figure 1). Nevertheless, retinol significantly improves pro-collagen I protein expression after 14 days, as well as Fibrillin 1 (FBN1) expression (Figure 1).

In parallel, we observed that PHE significantly increases SPARC expression after 7 days (+99%*) compared to the untreated control (figure 1). This interesting result is combined with a significant increase in pro-collagen I protein expression (+144%**) in the spheroids after 14 days. Additionally, we demonstrated that PHE is also able to potentialize protein expression of fibrillin 1 by ~400%***.

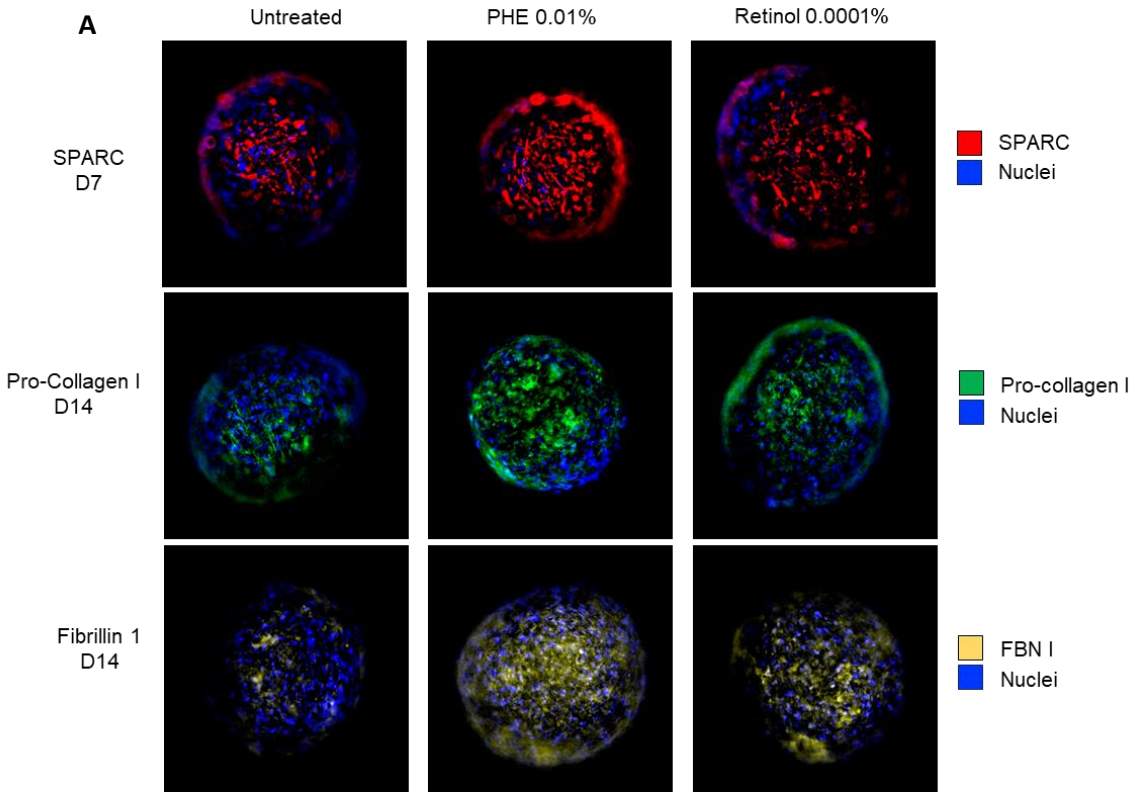

Figure 1: . Illustrative images of immunostaining for SPARC, pro-collagen I and Fibrillin 1 (FBN1) on dermal spheroids

5. Clinical efficacy of PHE compared to retinol

The objective of this first clinical study was to compare PHE to the widely used retinol anti-aging standard reference, on wrinkles and dermis density.

5.1 Global deep wrinkles and crow's feet reduction

We observed that PHE decreases global deep wrinkles with a cumulative effect over time (figure 2A). Wrinkles density variation is decreased of 0.23 at day 14 (corresponding to -5.1%, $p<0.1$) then 0.32 at day 28 (corresponding to -7.1%, $p<0.05$). Retinol shows a significant decrease only after 28 days of use, of 0.39 at day 28 (corresponding to -8.8%, $p<0.05$). Therefore, PHE can decrease wrinkles faster than retinol does, and with more positive responses of volunteers at day 28 (58.3% for retinol vs 66.7% for PHE). By an additional measurement, we determined that PHE decreases crow's feet wrinkles Rz parameter from 0.056 mm at day 0 to 0.053 mm at day 28 (corresponding to -5.3%, $p<0.05$) (figure 2B).

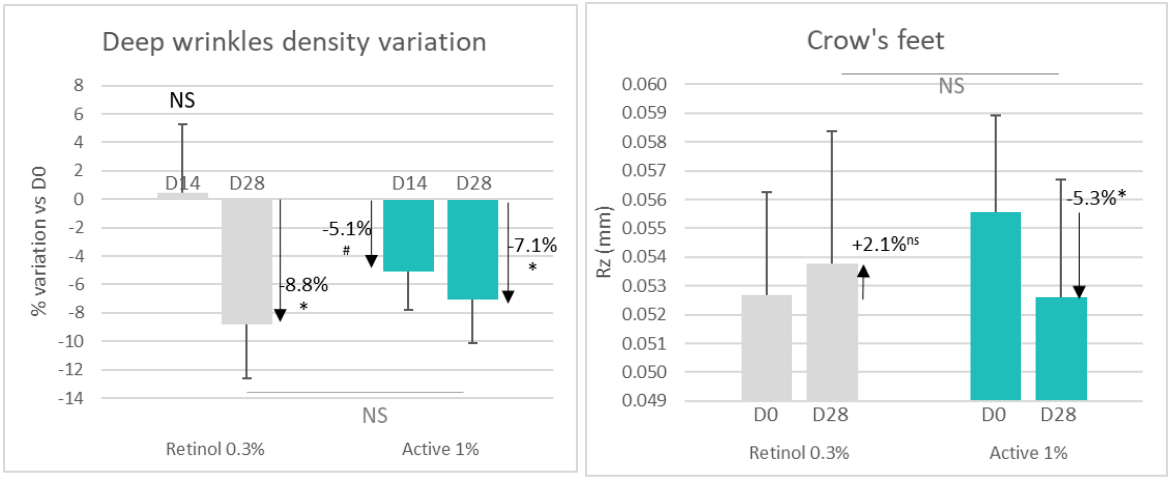

Figure 2: A. Global deep wrinkles variation by AEVA in all half-faces for Retinol and PHE. All are expressed as % variation of density related to face area. % variation are indicated in numbers (mean values vs Day 0); **B.** Measurement of crow's feet by AEVA for Retinol and PHE. All are expressed as Rz, the % variation are indicated in numbers (mean values vs Day 0). Statistics: *: $p<0.05$; #: $p<0.1$; ns: $p>0.1$.

5.2 *Improvement of dermis density*

We measured dermis density after 28 days of application. We showed that PHE improves the dermis density in 28 days of use (Figure 3). The density increases from 8.6 % at day 0 to 11.9% at day 28 with PHE (corresponding to +38.6%, p<0.01). In our experimental condition, we cannot observe any significant improvement with retinol.

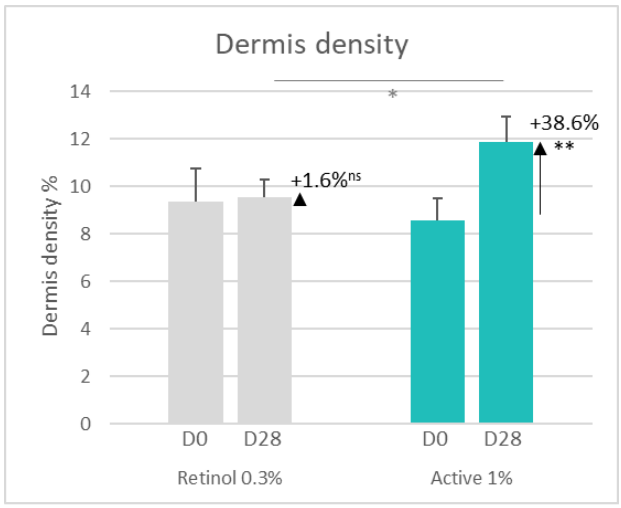

Figure 3: Dermis density measurement by echography for Retinol and PHE. All are expressed as % density. % variation are indicated in numbers (mean values vs Day 0). Statistics: *: p<0.05; **: p<0.01; ns: p>0.1.

6. Clinical efficacy of PHE compared to a natural retinol alternative, bakuchiol

The objective of this second clinical study was to compare PHE to the most popular natural retinol alternative, bakuchiol, and assessed clinical parameters such as skin firmness, brightness, and sagging.

6.1 PHE increases skin firmness

PHE improves skin firmness with a cumulative effect over time (Figure 4A) by decreasing R0 from 0.43 mm at day 0 to 0.37 mm at day 28 (corresponding to +12.6% firmness, p<0.001) then 0.34 mm at day 56 (+19.0% firmness, p<0.001) These effects are 6 times better than placebo, with highly significant differences at all timepoints (p<0.001). This positive effect on firmness is equivalent to the one of bakuchiol at both timepoints, as the difference between the effects of the 2 products are non-significant (p<0.001 for both timepoints, Figure 4B).

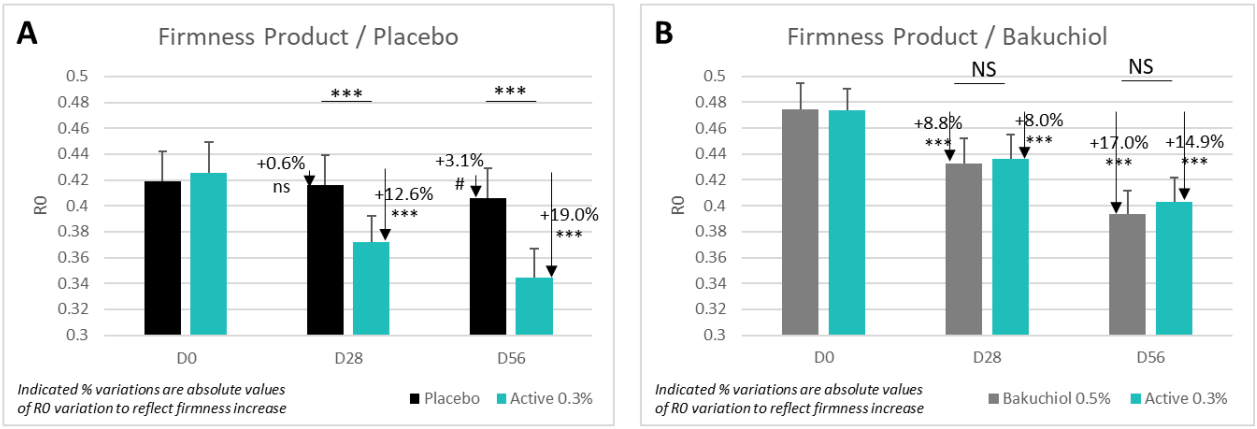

Figure 4: Results for firmness measurement by cutometry in both groups: PHE/Placebo (**A**) and PHE/bakuchiol (**B**). All are expressed as R0 values mean and absolute % R0 variation vs Day 0. Statistics: ***: p<0.001; #: p<0.1; ns: p>0.1.

6.2 Brightness enhancement by PHE

PHE is able to improve skin brightness (Figure 5A), as L* is significantly increased by +1.2 units (+1.9%, p<0.001) after 28 days of use, which is 9.5 times better than with placebo (p<0.001). Moreover, the efficacy of PHE is comparable to the one of bakuchiol (Figure 5B), as both present similar variation and are non-significantly different from each other (+2.0 units for bakuchiol corresponding to +3.5% (p<0.001) and +2.5 units for PHE corresponding to +4.3% (p<0.001)).

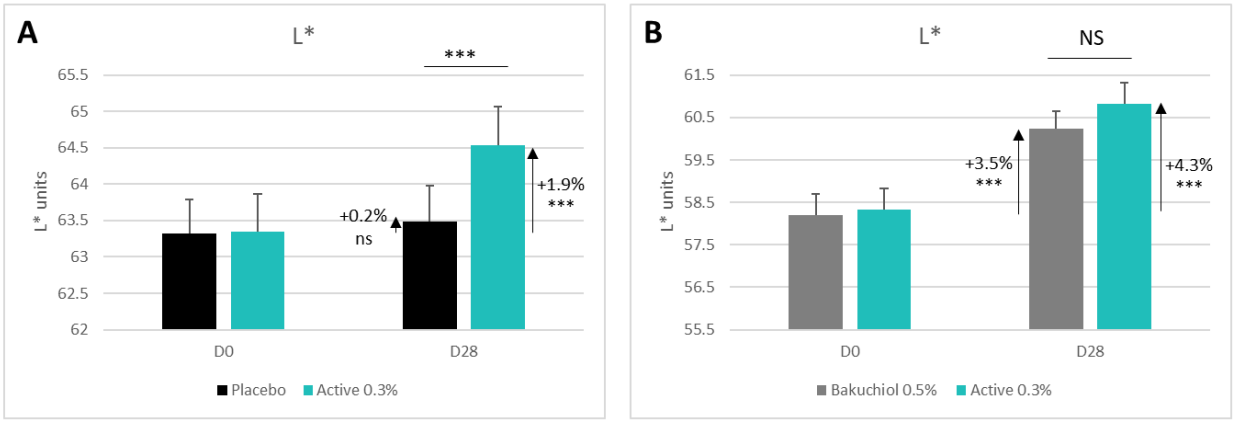

Figure 5: Results for brightness measurement (L*) in both groups: PHE/Placebo (A) and PHE/bakuchiol (B).

All are expressed as L units mean and % variation vs Day 0. Statistics: ***: p<0.001, ns: p>0.1.

6.3 Sagging improvement

PHE decreases sagging by 0.3 points (-10.7%, p<0.001) as soon as day 28, being 5 times better than placebo (p<0.001) (figure 6A). The most interesting part is that it presents a better efficiency than bakuchiol. While it shows a decrease of only 0.3 points (-8.9%, p<0.01), PHE is able to decrease the sagging on this panel by 0.5 points (-12.8%, p<0.001), being 1.4 times significantly better than bakuchiol (p<0.05). Moreover, PHE shows a positive effect on all panelists (100%) when bakuchiol acts only on 58% of volunteers (Figure 6B).

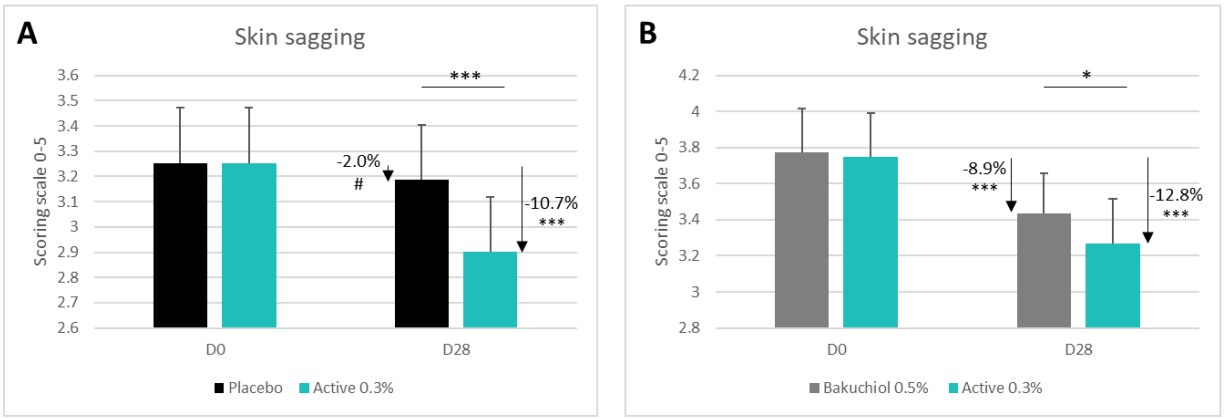

Figure 6: Sagging evaluation by scoring for PHE/Placebo (**A**) and PHE/bakuchiol (**B**). All are expressed as scoring scale value mean and % variation vs Day 0. Statistics: ***: p<0.001; *: p<0.05; #: p<0.1; ns: p>0.1.

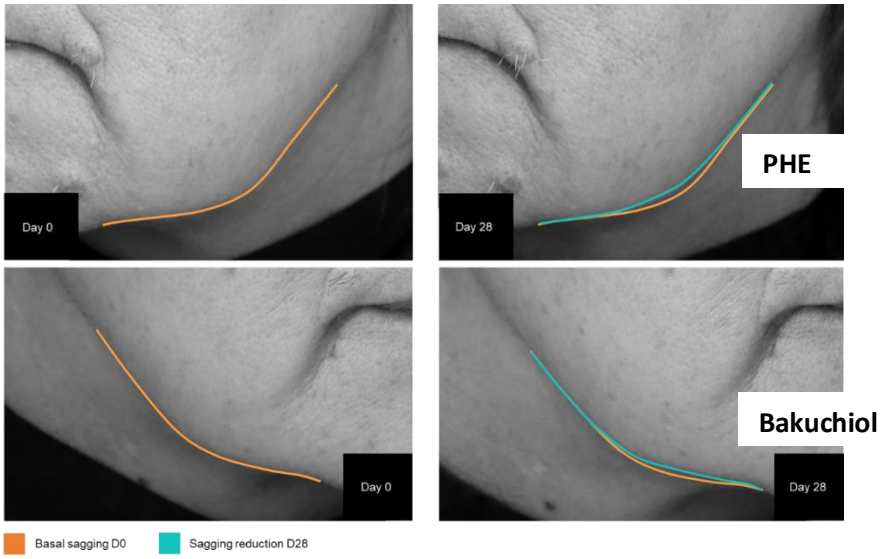

Figure 7: Illustration of sagging effect: illustrative pictures from volunteer 29 (60 years old).

Discussion

Retinol is the best-known anti-aging ingredient widely recognized for its effect on appearance of fine lines, wrinkles, uneven skin tone, and age spots. Retinoids boost collagen production and support overall smoother, more youthful-looking skin. However, they are also notoriously harsh, with common side effects including dryness, redness, peeling and irritation.

Therefore, more and more natural alternatives are being sought. The first of these alternatives was found around ten years ago with bakuchiol which has shown through numerous experimental and clinical studies its anti-aging effectiveness, similar to retinol, while being gentle for the skin [19]. Recently, cases of dermatitis have been reported and have rekindled the need to find new natural alternatives.

In our study, we used several experimental models to demonstrate the ability of a natural extract enriched with cyclic peptides (PHE) to improve retinoid metabolism. This complex metabolism involves numerous players such as dehydrogenases, cytochromes, nuclear receptors, etc. The fixation of the active metabolite, atRA, on its RAR type receptors therefore allows the modulation

of numerous genes both in the dermis and the epidermis. Using skin explant and human primary keratinocytes cultures, we showed the capacity of PHE to improve the global retinoid homeostasis by increasing several enzymes involved in the retinoic acid production and the control of intracellular content and also increasing RARγ expression, for an optimal effect of endogenous retinol use (figure 8).

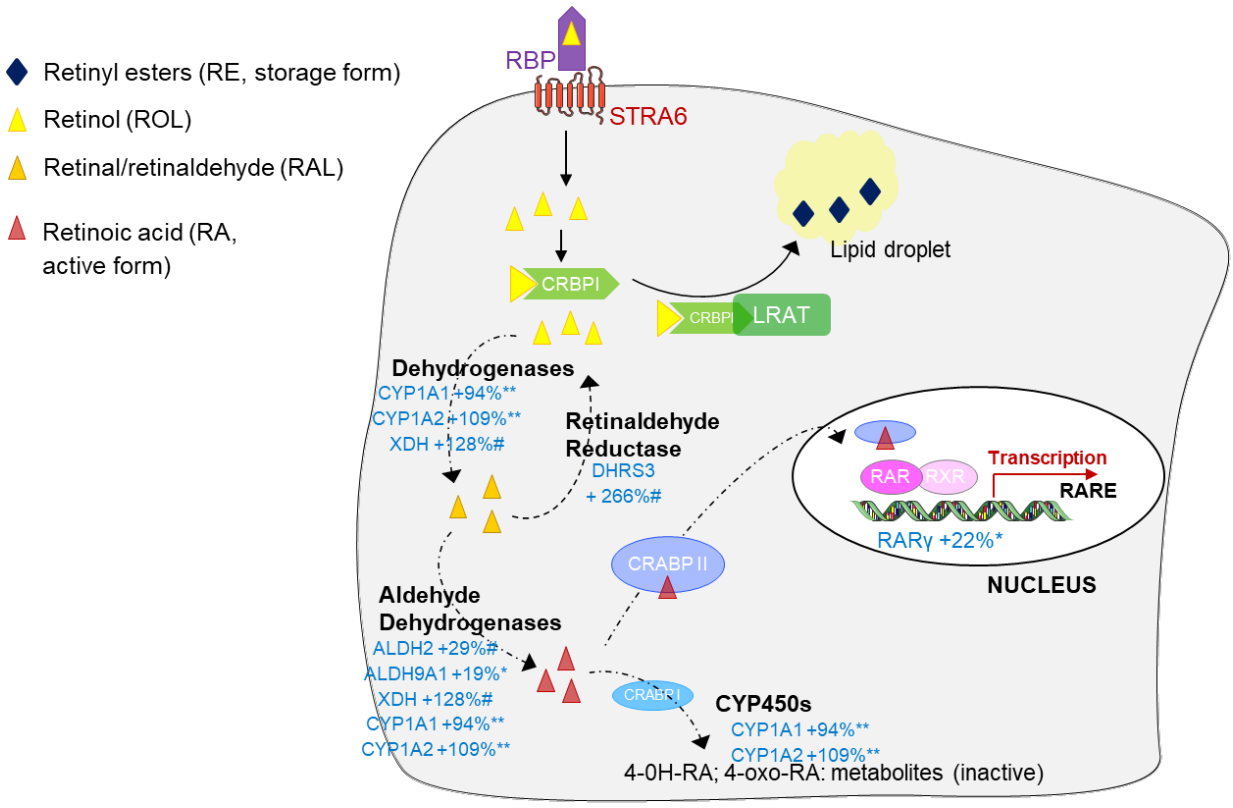

Figure 8: summary of PHE's effect on retinoid metabolism

In addition, we have highlighted a global anti-aging action of PHE on epidermis and dermis by results ranging from the gene (transcriptomic study on NHEK and NHDF), to the synthesis of pro-collagen I by fibroblasts but also on a 3D functional model of dermis.

Comparison with bakuchiol and retinol on NHDF indicates similar or greater pro-collagen I synthesis thanks to PHE. This effect is linked to the content in an original cyclopeptide: heterophyllin B. Moreover, in the spheroids model of dermis, we demonstrated the ability of PHE

to lead, with greater efficiency than retinol, the fibroblasts to produce their own matrix (collagen type I) but also the associated protein for the collagen and elastin network organization (SPARC and FBN1 respectively). These results highlight that PHE outperforms retinol for a more qualitative dermal matrix by improvement of collagen content and organization, as well as the elastic network organization.

This potential was confirmed by two independent clinical studies. One of them highlighting the potential of PHE to reduce wrinkles and improve the dermis density with a similar or even greater effect compared to retinol. The potential of our PHE extract is also confirmed versus bakuchiol on its potential to improve the firmness and radiance of the skin and to counteract skin sagging, better than bakuchiol.

The strong originality of our extract comes from its content in cyclopeptides, especially heterophyllin B, which are bioactive molecules naturally present in certain plants and involved in their defense against pathogens. Unlike linear peptides, they have a closed cyclic structure, making them more stable and resistant to degradation enzymes. Cyclic peptides may have greater potential as therapeutic agents than their linear counterparts [20] due to their superior cell membrane permeability [16] (example of Cyclosporin A by passive diffusion), better stability thanks to their three-dimensional cyclic structure, and greater selectivity for cell receptors, increasing their performance [21]. Thus, cyclic peptides seem to stand as new generation of peptides for cosmetic benefits.

Conclusion

In the skin anti-aging market, retinol is a reference for efficacy. However, numerous side-effects reports push the cosmetic industry to find more gentle solutions, having also more naturality. The first retinol alternative developed some years ago, bakuchiol, while having a well-

documented efficacy seems to suffer from some side effects in some cases. Thus, there is still a growing interest for natural, efficient, and gentle alternatives. Our study demonstrated that a natural cyclic peptide extract from *Pseudostellaria heterophylla* roots (PHE) can be this new alternative. It improves retinoid metabolism by increasing RARγ expression and activating retinoic acid production enzymes. Moreover, it exhibits superior anti-aging effects compared to retinol and bakuchiol, promoting collagen synthesis, dermal matrix organization, and reducing wrinkles in clinical studies. Thus, our extract, enriched in original cyclopeptide heterophyllin B, stands as a new, original, and natural alternative solution for retinol as an anti-aging active cosmetic ingredient.

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Conflict of interest statement

NONE

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