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Beyond Traditional Anti-Aging: A Tailor-Made Plant-Based Innovation to Answer Precisely Menopausal Skin Needs

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1. Introduction

Ageing is a natural biological process resulting from the impact of the accumulation of a wide variety of molecular and cellular damages over time. It is a multi-factorial process driven by both intrinsic (e.g.: time, genetic factors, hormones) and extrinsic (e.g., UV-exposure, pollution) factors [1]. For women, one of the key parameters in chronological ageing is the loss of hormones occuring at a certain period of life. This phenomenon, called menopause, can be defined, clinically, as the period in a woman's life after twelve consecutive months of amenorrhea. The progressive decline in hormones levels, from several years before (perimenopause) and during menopause, lead to significant endocrine and biological changes with a strong impact on skin quality and accelerated aging [2].

In women, serum levels of 17b-oestradiol, dehydroepiandrosterone (DHEA), progesterone, growth hormone (GH) and insulin-like growth factor 1 (IGF-1) significantly decrease with menopause. Particularly, the decline of 17b-oestradiol resulting in a hypo-estrogenic state, has been associated with a worsening of skin structure and function [3,4]. In early menopause, skin collagen levels decrease rapidly with a collagen reduction of approximately 30% in the first 5 years, followed by a further decline of 2% per year for the next 15 years [5]. In addition, estrogen deficiency causes sebaceous glands atrophy with decreased lipid production and excretion, exacerbating xerosis that is seen with aging [6]. The significant reduction in circulating estrogen levels also disrupts angiogenic processes, resulting in decreased microvascular density and altered network architecture [7]. Beyond physiological alterations, menopause symptoms (hot flashes, fatigue, anxiety, depression…) strongly impact women's quality of life. In a survey from 2023, women attending perimenopause and menopause reported a loss of self-esteem and social affects from their skin appearance [8]. Altogether,

these elements highlight that hormonal loss during menopause is a significant intrinsic contributor to skin aging in women and the need to consider these global phenomena.

Today, to counteract these global negative impacts of menopause, oral hormone replacement therapies exist. However, few solutions specifically target the skin health needs of menopausal women, which may not be fully addressed by conventional anti-aging . In this way, we introduce here a specific extract of *Leonurus japonicus* roots, developed using Plant-Milking™ technology. This innovative approach allows for sustainable access to original phytochemical compounds, resulting in an extract enriched in rare phenylpropanoid derivatives. Commonly known as Motherwort*, Leonurus japonicus* has long been recognized in traditional Chinese medicine as Yi Mu Cao, meaning, literally "beneficial herb for women" [9]. Modern research has confirmed several of its pharmacological properties, including benefits in managing gynecological disorders, cardiovascular protection and modulation of the nervous system [10]. We investigated the potential of this unique *Leonurus japonicus* extract (LJE) to counteract the menopause-associated skin alterations using hormone-dependent *in vitro* models specifically designed to assess key biomarkers of hormonal aging.

2. Materials and Methods

2.1. Leonurus japonicus enriched extract (LJE) preparation

This active ingredient was developed using Plant-Milking™ technology, an aeroponic cultivation method, to grow *Leonurus japonicus* plants. The resulting extract, named LJE, is enriched and standardized with key biomarkers, which are phenylpropanoid compounds, specifically lavandulifolioside, verbascoside, leonoside A, leonoside B, and leucosceptoside A. The production process involves cutting and air-drying the roots, followed by extraction using a solution of pure 1,3-propanediol containing 0.3% citric acid. The extract is then filtered to remove plant biomass and achieve clarity. This non-destructive recovery method enables multiple harvests from the same plants, making it a sustainable, recyclable process.

2.2. Effect of LJE on collagen metabolism in human dermal fibroblasts (NHDF) under basal and menopausal conditions

Basal model

Normal human dermal fibroblasts (NHDF) were isolated from human dermis of 4 different donors (35 – 40 – 44 and 48-years old female). The fibroblasts were seeded in a 24-well plate at the density of $2.5x10^4$ cells/well and allowed to adhere for 24h. Then, the cells were exposed 72 hours to LJE at 0.05% - 0.1% - 0.5% or Coenzyme Q10 at 0. 0015%.

Menopausal model

Normal human dermal fibroblasts (NHDF) were isolated from human dermis of 3 different donors (19 – 34 - 35 years old female). The fibroblasts were seeded in a 12-well plate at the density of $5x10^4$ cells/well and allowed to adhere and growth until reaching confluence for 6 days. Then, stimulation of collagen synthesis was induced for 4 days with growth factors (50ng/mL of FGFβ (Fisher, 17800393), 50ng/mL of insulin (Sigma, I2643), 3mM of L-glutamine (Sigma, 59202C), 1µg/mL hydrocortisone (Sigma, H0888) and 50µg/mL of L-ascorbic acid (Sigma, A4544) and non-menopausal hormone levels (Table 1). This step enables to enhance extracellular matrix production with collagen composition close to that of young women's dermis. A serum deprivation phase was then performed to stabilize cell activity for 24h. Finally, cells were treated for 72h with hormonal concentration of menopausal women (Table 1) with addition or not of LJE at 0.05% - 0.1% - 0.5%. In positive control, certain cells were treated with hormonal concentration of non-menopausal women (Table 1).

Hormones	Non-Menopausal (20-50yo)	Menopausal (50-80yo)
	NM	M
17β-estradiol	750pM	6pM
Progesterone	60nM	0.6nM
Dehydroepiandrosterone	20nM	0.2nM
Growth hormone	5ng/mL	0.15ng/mL
Insulin-like growth factor-1	200ng/mL	10ng/mL

Table 1. Average concentrations of hormones in serum of non-menopausal and menopausal women, which were added to cell culture. Concentrations were calculated as the averages of concentration in blood serum in non-menopausal women (20–50 years old) and in menopausal women (50–80 years old) [3,4].

At the end of the incubation period, supernatants were collected for ELISA analysis of pro collagen 1 (Biotechne, #DPCA00) and MMP-1 (Biotechne, #DMP100) production following supplier protocol. The cells were lysed with NaOH 1N to quantify total proteins following Bradford assay protocol. Analysis was done by calculating the ratio of pro collagen 1 or MMP-1 (pg/mL) on total proteins (µg/mL) to obtain normalized value for each well. The mean and standard deviation for each condition is then calculated.

2.3. Effect of LJE on lipid production in human sebocytes under non-menopausal and menopausal conditions

Human sebocytes from Cellprogen (#36079-01) at different passages were seeded in a 96-well plate at the density of $1.5x10^4$ cells/well and allowed to adhere for 24h. Then the cells were treated for 48h with hormonal concentration of menopausal or non-menopausal women (Table 1) with addition or not of LJE at 0.01% - 0.05% - 0.1%.

At the end of the incubation period, the cells were fixed with paraformaldehyde 10% and stained with BODIPY dye at 5µM (Sigma, #790389) for lipid droplets and Hoechst at 2µg/ml (Invitrogen, # H3570) for nuclei, for 30 minutes. Images were captured with Nikon Eclipse Ti2 fluorescence microscope on FITC (Bodipy) and DAPI (nuclei) channels with the X20 lens (3 images/well). Quantification was then

performed with ImageJ software: Bodipy staining area and nucleus number. Analysis was done by dividing the lipid droplets area by the number of nuclei to obtain an average of total lipid content by cell. The mean and standard deviation for each condition is then calculated.

2.4. Effect of LJE on microvascular network formation in human umbilical vein endothelial cells (HUVEC) under menopausal conditions

Pool of human umbilical vein endothelial cells (HUVEC) from 10 donors (Promocell, # C-12203) at different passages were used for these experiments. Briefly cells were seeded onto a Matrigel (Sigma, #CLS356237-1EA) matrix in 96-well plates at the density of $2x10^4$ cells/well and treated or not either with LJE at 0.05% and 0.1% or with the biomarkers, i.e. total phenylpropanoid (PPG), at dose equivalent to the one contained in LJE, in addition to hormonal concentration of menopausal women (Table1). In positive control, certain cells were treated with hormonal concentration of non-menopausal women condition (Table 1). Cells were incubated for 16h at 37°C, 5% CO_2 to allow microvascular network formation.

At the end of the incubation period, images were captured with Biotek Cytation3 microscope in phase contrast mode with the X4 lens (4 images/well). Microvascular network formation was characterized by image analysis using Angiogenesis analyzer tool [11] on ImageJ software. Number of master junctions (junctions that link at least three segments), master segments (pieces of tree delimited by two master junctions) and meshes (areas enclosed by segments) were calculated by the software and used as parameters to analyze the network. The mean and standard deviation for each condition is then calculated.

2.5. Statistical analysis

All results were expressed as percentages of control condition (untreated, menopausal or non-menopausal) and were submitted to ordinary one-way ANOVA, corrected Brown-Forsythe and Welch ANOVA or Kruskal-Wallis comparisons test depending on normality and variance check. The statistical significance value is $p<0.05$ (#$p<0.1$, *$p<0.05$, **$p<0.01$, ***$p<0.001$).

3. Results

3.1. LJE promotes natural dermal matrix synthesis and counteracts dermal atrophy occurring at menopause onset

As expected Coenzyme Q10, a well known anti-aging molecule, significantly stimulated pro-collagen 1 synthesis at 0.0015% (17.5µM) of incorporation ($1/100^{th}$ of the clinical dose), with an increase of 67% (Figure 1). This result is consistent with previous findings reported in the literature [12]. Similarly, LJE demonstrated a significant, dose-dependent, increase in pro-collagen 1 production under basal condition. At concentrations of 0.1% and 0.5%, LJE showed comparable efficacy to Coenzyme Q10,

with no statistically significant difference between the treatments (p-value = 0.387 at 0.1% and p-value = 0.985 at 0.5%).

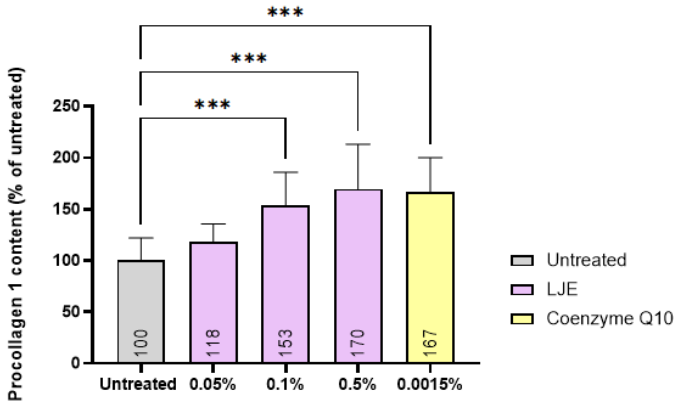

Figure 1. Production of pro-collagen 1 in human dermal fibroblasts (NHDF). NHDF from 5 different donors (35yo, 40yo, 44yo and 48yo) (N=4 – n=4) treated or not with *Leonurus japonicus* extract (LJE) or the benchmark coenzyme Q10. Results are expressed in percentage of variation compared to untreated control condition. Statistics: ANOVA test vs control, * p<0.05, ** p<0.01, *** p<0.001.

The hormonal decline associated with menopause significantly impaired skin matrix homeostasis, leading to a 37% decrease in pro-collagen 1 production and a 178% increase in MMP-1 secretion compared to the non-menopausal condition (Figure 2). These results are consistent with existing literature and validate the relevance of our hormonal aging model. Under menopausal stress, treatment with LJE significantly enhanced pro-collagen 1 synthesis by 43% and 84% at 0.1% and 0.5%, respectively (Figure 2A), recovering to the non-menopausal state (no significant difference vs. NM, p-value = 0.699; 0.649 and 0.188 at 0.05%; 0.1% and 0.5% respectively). In parallel, LJE significantly reduced MMP-1 secretion by 46% at both concentrations (Figure 2B), again restoring levels comparable to those observed under non-menopausal conditions (no significant difference vs. NM, p-value = 0.754 and 0.538 at 0.1% and 0.5% respectively).

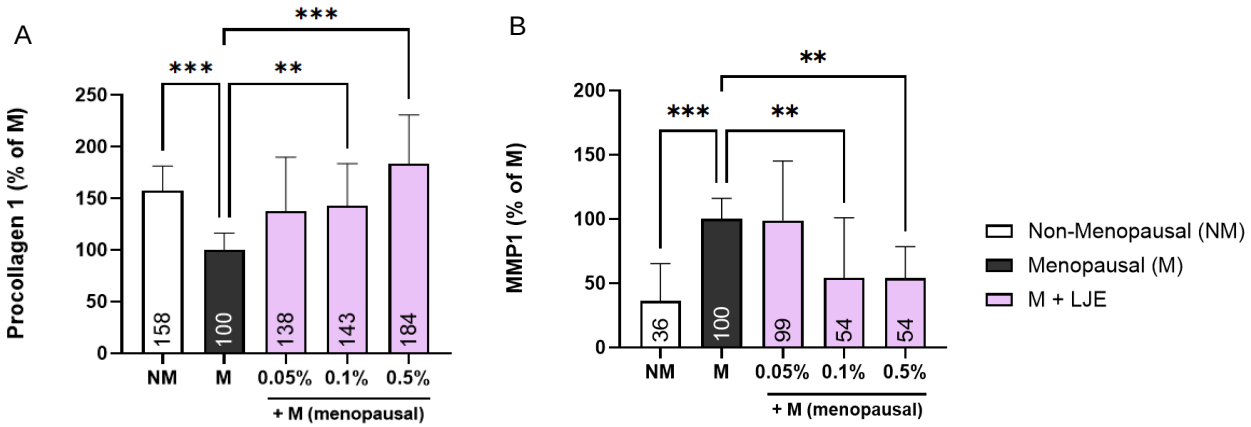

Figure 2. Production of pro-collagen 1 (A) and MMP-1 (B) on menopause-stressed human dermal fibroblasts (NHDF) NHDF from three different donors (19yo, 34yo and 35yo) (N=5 – n=4) treated with Non-menopausal (NM) or Menopausal (M) hormonal cocktails ± Leonurus japonicus extract (LJE). Results are expressed in percentage of variation compared to M control condition. Statistics: ANOVA test vs control, * p<0.05, ** p<0.01, *** p<0.001.

3.2. LJE induces sebogenesis specifically under menopausal condition

Menopausal hormonal decline significantly deregulated lipid metabolism, resulting in a reduction of 64% in lipid content compared to the non-menopausal condition (Figure 3). Under menopausal conditions, LJE treatment demonstrated significant efficacy in restoring lipid production with all tested concentrations, reaching an increase of +77% in lipid content at 0.1%.

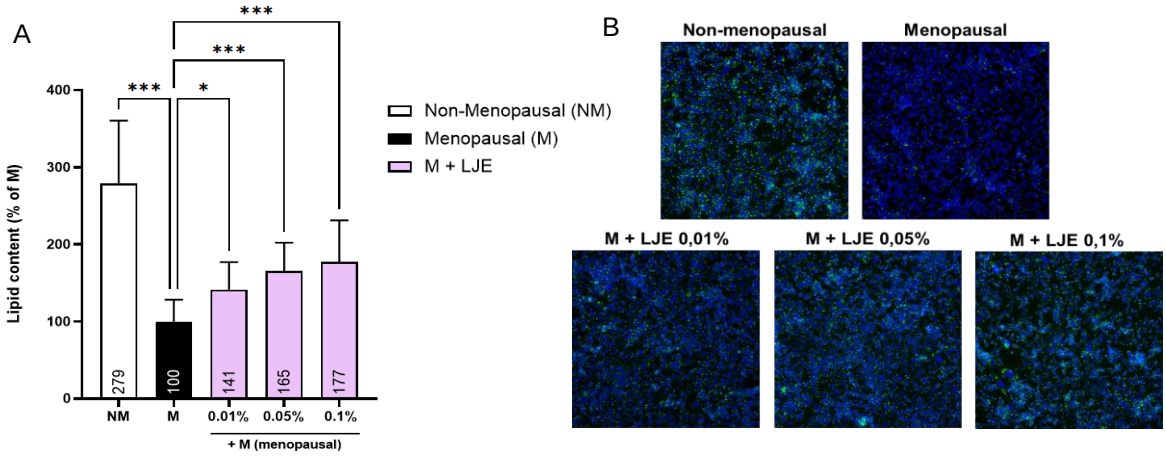

Figure 3. Production of lipid droplets on menopause-stressed human sebocytes. Human sebocytes (N=4 – n=4) treated with Non-menopausal (NM) or Menopausal (M) hormonal cocktails ± Leonurus japonicus extract (LJE). (A) Lipid content in human sebocytes. Results are expressed in percentage of variation compared to M control condition. Statistics: ANOVA test vs control, * p<0.05, ** p<0.01, *** p<0.001. (B) Microscopical observation (x20) of human sebocytes stained with BODIPY (green for lipid droplets) and DAPI (blue for nucleus).

Under physiological hormonal conditions characteristic of non-menopausal women (representative of young skin), LJE treatment did not induce any significant increase in sebaceous lipid production highlighting its specific activity toward menopause-induced deficiencies (Figure 4). The lipid content remained comparable to baseline non-menopausal levels, with no statistically significant variations observed between LJE-treated and non-menopausal condition.

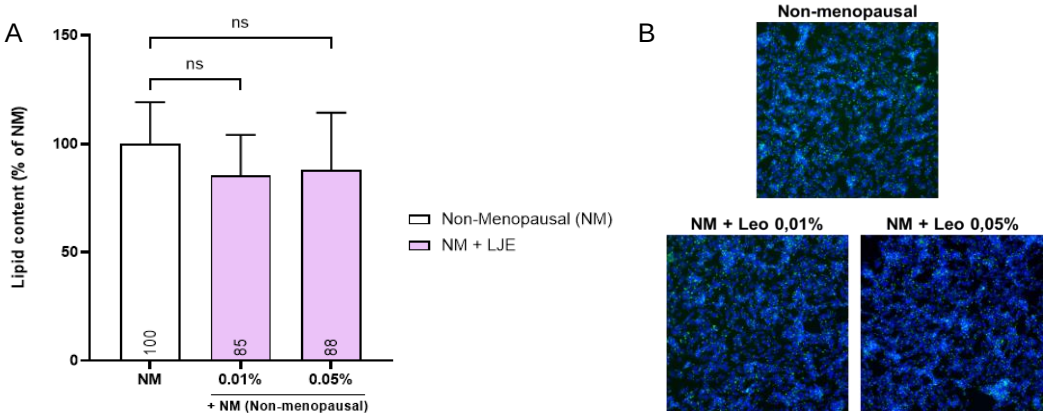

Figure 4. Production of lipid droplets on human sebocytes under non-menopausal (i.e. young) conditions. Human sebocytes (N=3 – n=4) treated or not with Non-menopausal (NM) ± Leonurus japonicus extract (LJE). (A) Lipid content in human sebocytes. Results are expressed in percentage of variation compared to NM

control condition. Statistics: ANOVA test vs control, * p<0.05, ** p<0.01, *** p<0.001. (B) Microscopical observation (x20) of human sebocytes stained with BODIPY (green) and DAPI (blue).

3.3. LJE and its biomarker improve microvascular network integrity under menopausal condition

Drop in hormonal levels at menopause significantly altered microvascular network formation, as evidenced by reductions in key structural parameters compared to non-menopausal conditions: master junctions decreased by 28%, master segments by 31%, and meshes by 41% (Figure 5). Under menopausal conditions, LJE significantly enhanced microvascular network formation. From 0.05% of incorporation, LJE restored mesh numbers to levels comparable with the non-menopausal condition (Figure 5C), while going slightly beyond non-menopausal baseline values for both master junctions and segments (Figure 5A-B). This pattern does not reflect a larger network or a pro-angiogenic effect but rather indicates a denser, more complex vascular architecture with increased ramification, suggesting the formation of a more functional and mature network. The same trend was observed with total biomarkers (PPG), demonstrating substantial increases in network formation, up to +138% in junctions' formation, +117% in number of master segments and +85% in number of meshes at 0.05%. Notably, these improvements showed comparable efficacy with LJE with no significant differences at equivalent concentrations on the three parameters. These results suggest that the biomarkers, total phenylpropanoids, are key drivers of the observed activity.

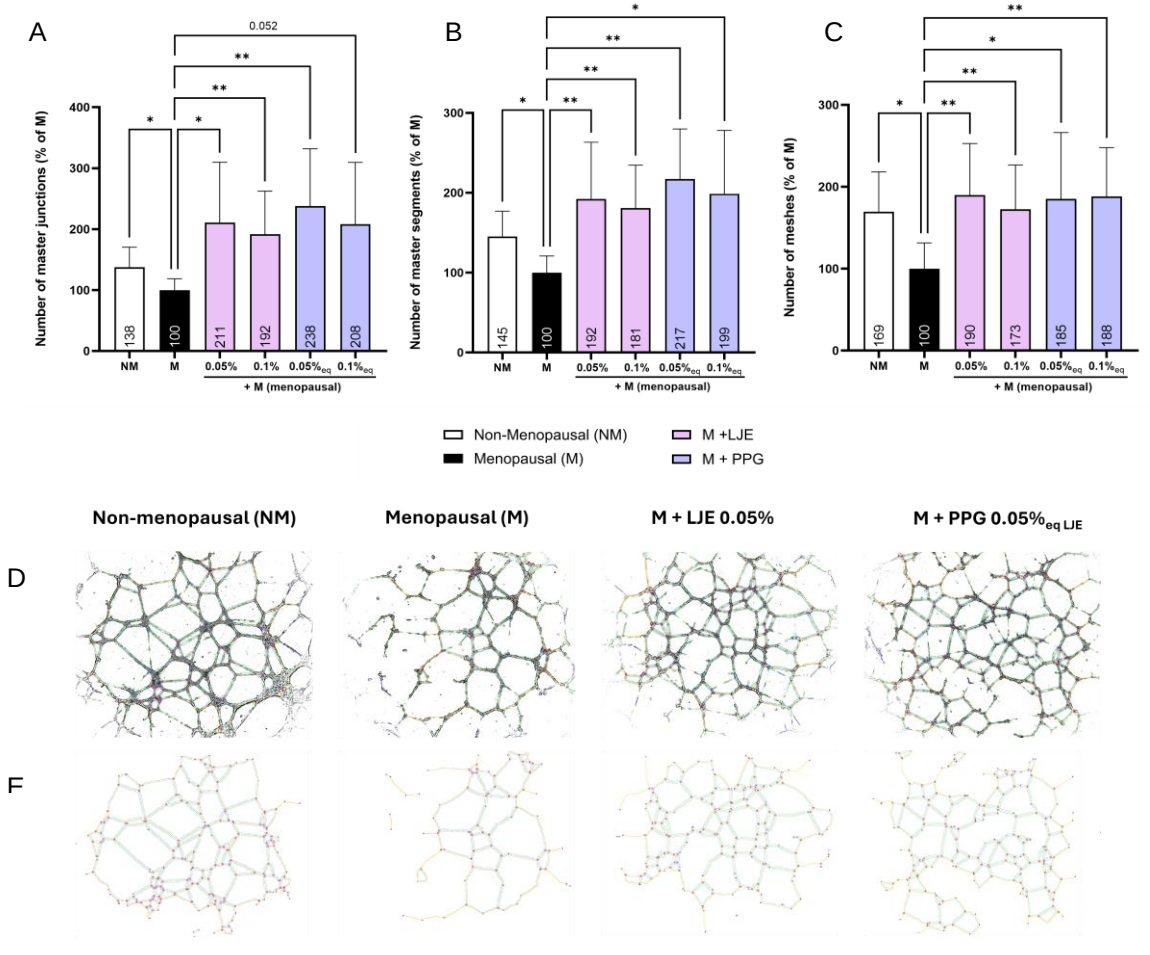

Figure 5. Microvascular network formation on menopause-stressed human endothelial cells (HUVEC). HUVEC (N=3 – n=4) treated with Non-menopausal (NM) or Menopausal (M) hormonal cocktails ± Leonurus japonicus extract (LJE) or the total biomarkers (PPG) at equivalent concentrations to the one contained in LJE. (A) Number of master junctions, (B) number of master segments and (C) number of meshes of the microvascular networks. Results are expressed in percentage of variation compared to M control condition. Statistics: ANOVA test vs control, # $p<0.1$ * $p<0.05$, ** $p<0.01$, *** $p<0.001$. (D) Microscopical observation (x4) of HUVEC microvascular network. (E) Detection of master segments (Yellow) meshes blue (sky), Junctions (blue), master junctions (junction surrounded by red) with Angiogenesis analyzer tool on ImageJ software.

4. Discussion

The skin contains hormonal receptors (mainly estrogen receptors) distributed throughout its structures, including the epidermis, dermis, hair follicles, and sebaceous glands, responsible for critical functions [13]. In consequence, the significant decline in sex steroid hormones at menopause induces profound alterations in skin physiology. The menopausal hormonal deficit triggers a cascade of changes: skin thinning, vascular fragility, disruption of matrix homeostasis, reduced glycosaminoglycan content, deregulated sebum production and altered barrier function [5,8]. Conventional anti-aging solutions are often insufficient when it comes to addressing the specific skin challenges associated with menopause. To meet this unmet need, our work focused on three key parameters, characteristic of menopause impact on skin to define a tailor-made solution, based on a natural plant extract and designed to counteract these harmful effects.

First, dermal atrophy and loss of firmness are one of the major concerns in menopausal women. The decline in hormones levels disrupts fibroblast function, significantly reducing procollagen 1 production while increasing matrix degradation [14]. Our plant extract, LJE, developed using a unique process, shows efficacy comparable to Coenzyme Q10 in stimulating pro-collagen 1 production in basal condition. LJE also demonstrates significant efficacy in stimulating pro-collagen 1 production and reducing MMP1 secretion in menopausal condition. The results indicate the ability of LJE to globally improve pro-collagen 1 synthesis and more specifically to counteract the effect of menopause on collagen metabolism by acting on fiber synthesis and matrix remodeling dysfunction. This profile of action is relevant to a strong skin anti-aging compound.

Second, sebaceous glands activity strongly decreases at menopause. In consequence, skin has an altered protective lipid layer leading to drier and less hydrating skin [3]. Notably, LJE enhances sebogenesis in menopausal condition helping to recover the loss of sebum production but does not cause sebum overproduction in non-menopausal (i.e. young) condition. This observation suggests that LJE's lipogenic effects are specifically targeted toward counteracting menopause-induced deficits rather than inducing non-physiological stimulation of lipid synthesis in homeostatic conditions.

Finally, reduction in circulating hormones is associated with endothelial dysfunction. Microvascular network formation is altered resulting in impaired oxygen delivery, nutrient distribution, and metabolic

waste removal [7,15]. We demonstrated that our plant extract enriched in unique biomarker molecules improves both the architectural complexity and density of microvascular network. This improvement supports optimal vascular function, promoting better oxygenation and nutrient delivery. Such effects are particularly valuable for enhancing overall skin quality and counteracting the vascular decline associated with menopause, helping to maintain skin vitality and radiance.

Leonurus japonicus extract (LJE) demonstrates multi-targeted efficacy in addressing key hallmarks of menopausal skin aging. Through its ability to enhance dermal matrix homeostasis, restore sebaceous lipid production, and improve microvascular network architecture, LJE presents a promising natural solution for menopausal skincare formulations. To further validate these promising *in vitro* findings, the next phase of research will include a comprehensive clinical study on a multiethnic cohort of peri- and early menopausal women. In parallel, Coenzyme Q10 will be tested as a benchmark to compare efficacy and further highlight the relevance of our targeted approach. This clinical assessment will evaluate well-aging parameters specifically identified through preliminary internal consumer survey on menopausal skin needs. Physiological parameters evaluation will focus on key concerns including skin dryness, dermal structural integrity, and radiance. Importantly, this study will extend beyond physical parameters to assess the psychosocial impact of the active, measuring improvements in well-being, self-esteem, and social confidence related to skin appearance. This holistic approach aligns with the growing recognition that effective menopausal skincare solutions must address both the physiological and psychological aspects of skin aging during this significant life transition period [8].

5. Conclusion

These findings demonstrate LJE unique ability to mitigate the complex cascade of skin alterations induced by menopausal hormonal decline. Through the implementation of tailored made hormone-dependent models, this study establishes an innovative and targeted approach to address hormone-mediated skin aging. LJE represents a shift from a conventional anti-aging approach, offering a solution specifically designed for menopausal skin needs. Beyond its physiological benefits, this targeted approach addresses the broader impact of menopausal skin changes on women well-being and self-esteem. This holistic approach positions LJE as a new benchmark in the development of specialized menopausal skincare solutions.

6. References

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