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CLINICAL APPROVED PERFORMANCE ON ANTI-AGING BASED ON COMBINED MECHANISMS OF ANTI-GLCATION AND ECM&DEJ REMODELING

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1. Introduction

Aging is a natural process that involves the gradual decline in the body's physiological function and accumulation of damage [1]. One of the most obvious aging signs is skin change. With the process of aging, the skin loses its elasticity, its ability to retain moisture and capacity for self-repair, leading to signs of aging including fine lines, wrinkles and sagging [2].

The dermis contains a large quantity of extracellular matrix (ECM) components, including collagen, elastin, and glycosaminoglycans. A reduction in the ECM further speeds up the development of wrinkles and the sagging of the skin [3]. And at the dermal-epidermal junction (DEJ), thickening of the stratum corneum (SC), thinning of the dermis and epidermis and loss of Collagen IV and VII are also the factors contributing to wrinkle formation [3].

Glycation is a non-enzymatic chain of reactions between proteins and sugars that leads to the production of advanced glycation end products (AGEs). It can be found ubiquitously in humans and tends to accumulate extensively in tissues with aging [4]. Many different AGEs have been identified, such as pentosidine, glucosepane, Nε-carboxymethyl-lysine (CML), and Nε-carboxy-ethyl-lysine (CEL) [5]. At the cutaneous level, these AGEs accumulate in skin and bind with collagen and elastin, causing the loss of elasticity and firmness, which is a critical factor in skin aging [2]. Furthermore, another approach is through a sequence of signaling pathways mediated by AGEs binding to their receptors and subsequently regulates the expression of genes [6]. RAGE is the most studied receptor for AGEs [7]. It was shown that AGEs inhibit

proliferation and promote apoptosis of dermal fibroblasts, a process that is partially mediated by RAGE and associated with multiple pathophysiological mechanisms [8].

Various strategies have been developed to address skin anti-aging, here we aimed to achieve enhanced effect on anti-aging via combined mechanisms of inhibition of glycation process and ECM&DEJ remodeling. Flavonoids are polyphenol compounds that have shown significant effects on the inhibition of protein glycation and AGEs formation by multiple mechanisms such as ROS inhibition, capturing reactive amino groups and chelating with trace metal ions that catalyze glycation [9]. In this research, 3 plant flavonoids (Blueberry fruit extract, Pomegranate fruit extract and Cassia Alata leaf extract) were selected based on synergetic anti-glycation effect, combined with C-xyloside, which many studies have provided compelling evidence supporting its efficacy on improving skin quality and decelerating the skin aging process via restoring ECM and DEJ structure. we evaluated the combination of these actives on anti-glycation efficacy and the improvement performance of skin elasticity, sagging and wrinkles at in-vitro, ex-vivo and clinical level.

2.Materials and methods

2.1. An In-tube AGEs inhibition test was initiated to evaluate the anti-glycation efficacy of Blueberry fruit extract, Pomegranate fruit extract and Cassia Alata leaf extract in combination or individually. A bovine serum albumin (BSA) and glucose solution was used to mimic the glycation process that occurs in the skin, with 40mg/mL BSA and 120mg/mL glucose in each reaction system. A positive control (aminoguanidine hydrochloride) and a negative control (PBS replacing sample) were included. The test samples or control were mixed in the glycation reaction system and incubated at 55°C for 40 hours to allow the glycation re-action to proceed and AGEs formation. After incubation, the AGEs fluorescence intensity of was measured using a fluorescence microplate reader at an excitation wavelength of 370nm and an emission wavelength of 440nm. The AGEs inhibition rate was calculated by the measurement of AGEs fluorescence intensity.

2.2. Evaluation of the protective effects of the serum against UV and/or methyl glyoxal (MG) induced glycative stress in ex vivo human skin. 67 human skin explants were prepared on an

abdominoplasty coming from a 50-year-old woman with a Fitzpatrick skin phototype II. On day 0, the explants were kept in survival in BEM culture medium at 37°C in a humid, 5%-CO2 atmosphere. Test samples were topically applied on skin surface of the corresponding explants at the rate of 2µL per 1cm² explant (≈ 2mg/cm²) on day 0 (D0), D2, D5 and D7. The control explants T did not receive any treatment except the renewal of culture medium. The BEM culture medium was half renewed (1ml per well) on D2, D5 and D7. In order to induce glycation, the methylglyoxal solution was incorporated in the BEM culture medium at a final concentration of 500 µM on D2, D5 and D7. On D5 and D7, the culture media of “U” and “GU” batches were re-placed by HBSS (Hank’s Balanced Saline Solution; 1 mL per explant) and irradiated using a UV simulator Vibert Lourmat RMX 3W with a dose of 9 J/cm^2 of UV-A corresponding to 2 MED (minimal erythemal dose) on a skin with a phototype II. The unirradiated batches were kept in HBSS in the dark. At the end of the irradiation, all the explants were put back in 2 mL of BEM medium. Pentosidine, CML and RAGE.

2.3. The randomized controlled clinical study was conducted in China between September to December in 2024. This study followed the guidelines of the declaration of Helsinki. Volunteers gave their informed consent to participate in the study. The study was approved by the local ethic committee (ethic approval SECCR2024-141-01). 45 Chinese women, all skin types, aged 30–70 were recruited. They all presented with mild and higher score on skin tone yellowness adjusted by dermatologist based on Atlas. The BMI (Body Mass Index) was between 18.5 and 23.9, with visible wrinkles according to investigator’s confirmation. Subjects were instructed to apply the serum on full face and one side of randomized upper arm twice a day (morning and evening), had images taken, instrumental measurement at the time points Baseline(T0), Week 4 (T4w), Week 8(T8w). Skin autofluorescence (SAF) value was measured by The AGE Reader mu (DiagnOptics Technologies B.V., Groningen, Netherlands). The facial photo is captured by VISIA-CR (Canfield, New Jersey) on the front, left and right sides with all lighting modes.

2.4. Clinical study was conducted in China, between March to June in 2024. The research was in accordance with the guidelines of the Helsinki Declaration, with approval of the local Ethics

Committee (ethic approval FSEC2024-18-01). 42 Chinese women, all skin types, aged 30–60 were recruited, 50% of them self-declared as having sensitive skin. They all presented with corresponding severity for the attributes evaluated by dermatologist: ptosis of the lower part of the face (Skin Aging Atlas, 1≤grade),elasticity (Griffith, 3≤ grade≤6), forehead wrinkles (Skin Aging Atlas, 1≤ grade≤4), nasolabial fold(Skin Aging Atlas, 1≤ grade≤4), underneath eye wrinkles (Skin Aging Atlas, 2≤ grade≤5), crow's feet wrinkles (Skin Aging Atlas, 1≤ grade≤4), wrinkle around the corner of the lip (Skin Aging Atlas, 1≤grade≤4). Subjects were asked to apply the serum twice a day (morning and evening), had images taken, dermatologist assessment and instrumental measurement at the time points Baseline(T0), 15min after application (Timm), Week 1 (T1w), Week 4 (T4w), Week 8(T8w) and Week 12(T12w). Skin's elastic properties was measured by Cutometer® Dual MPA 580 (Courage + Khazaka, Germany), specific wrinkle parameters (forehead wrinkles, nasolabial fold) were analyzed by PRIMOS-CR (Canfield, America). The facial image is captured by VISIA-CR (Canfield, America) on the front, left and right sides with all lighting modes.

2.5. The study was conducted in Canada, between June to September in 2024. This study strictly followed the guidelines of the declaration of Helsinki and was approved by the local ethic committee (ethic approval 2024-3510-18255-3). 84 subjects with multiple ethnicities (Fitzpatrick skin type I-VI), all skin types, aged 30–60 were recruited, 50% of them self-declared as having sensitive skin. They all presented with mild to moderate level (score of 3-6 according to 0-9 scale) for the attributes evaluated by dermatologist: Skin sagging, elasticity, firmness, crow’s feet wrinkle, underneath eye wrinkle, nasolabial wrinkle, forehead lines, marionette wrinkles, neck wrinkles, global winkles, radiance. Subjects were asked to apply the serum twice a day (morning and evening), the subjects had images taken, dermatologist assessment and instrumental measurement at the time points Baseline(T0), Week 1 (T1w), Week 4 (T4w), Week 8(T8w) and Week 12(T12w). The facial image is captured by VISIA-CR (Canfield, America) on the front, left and right sides with all lighting modes.

3. Results

3.1. In-tube AGEs inhibition test: The combinations of three active ingredients Blueberry fruit extract, Pomegranate fruit extract and Cassia Alata leaf extract showed synergistical AGEs inhibition rate at 14.27%, which is statistically significantly ($p < 0.05$) higher than any single active, also the combination of any two actives.

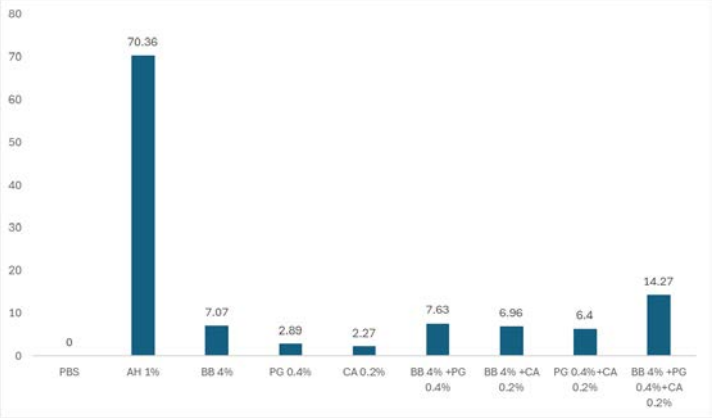
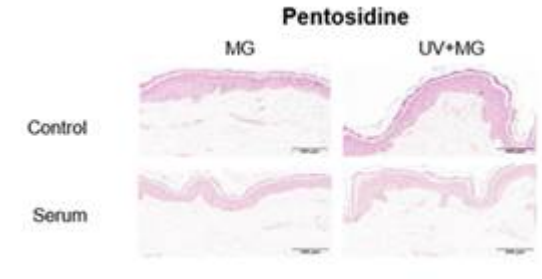
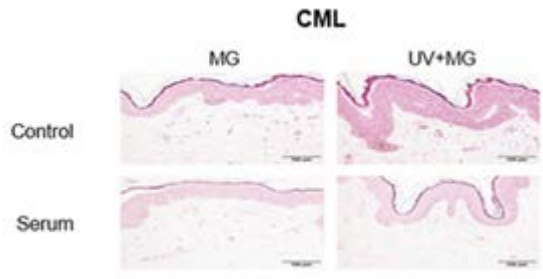


Figure 1. AGEs Inhibition Rate (%). PBS – negative control, AH - aminoguanidine hydrochloride as positive control, BB - Blueberry extract. PG - Pomegranate extract, CA - Cassia Alata Leaf Extract

3.2. CML and pentosidine are often used as biomarkers for glycation processes [4]. Through ex-vivo skin models, the formulation of serum significantly ($p < 0.05$) reduced skin glycation biomarkers induced by Methyl Glyoxal and UV, including pentosidine, CML, and RAGE, with 23%, 43% and 14% reduction rate at epidermis.



(a)



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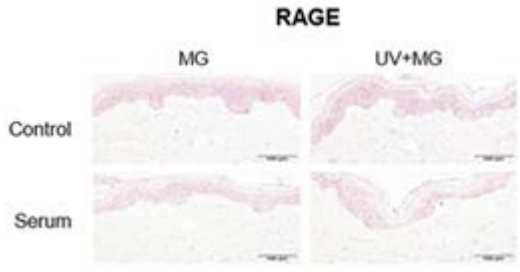

(c)

Figure 2. Immunostaining on ex vivo human skin. (a) Pentosidine (b) CML (c) RAGE

3.3. A total of 43 subjects completed the 8-week treatment. Compared with baseline on face, SAF value significantly ($p < 0.05$) decreased, which showed significant improvement on anti-glycation efficacy after 8 weeks repeated application. When compared with the blank control area, the change value of the applied area on upper arm had a significant difference in SAF value.

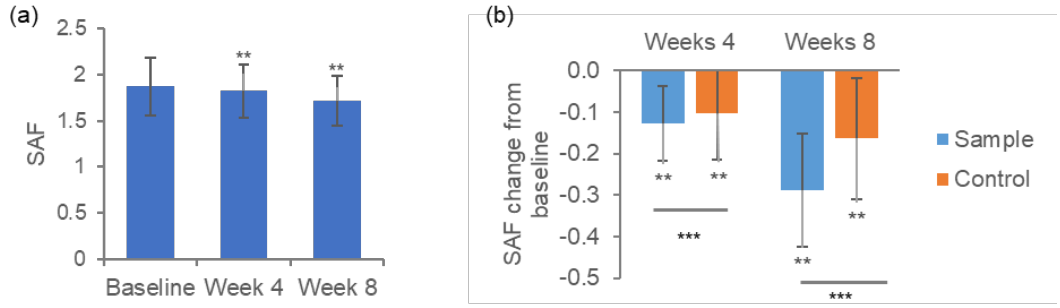

Figure 3. SAF measurement results by AGE READER. (a) SAF value on facial cheek area before and after 4 ,8 weeks of serum application. (b) Changes from baseline in SAF value on upper arms in both serum group and blank control group. **$p<0.05$ versus baseline. ***$p<0.05$ between two groups.

3.4. The study was finally conducted in a group of 42 Chinese women. Compared with clinical grading baseline, the serum delivered significant ($p < 0.05$) efficacy on improving skin quality related attributes: firmness, elasticity, sagginess and skin wrinkle related attributes: marionette line, forehead wrinkle, nasolabial fold, crow's feet wrinkle, underneath eye wrinkle after 12 weeks repeated applications. Results in China were highlighted that the count of forehead and nasolabial wrinkles measured by PRIMOS significantly decreased at week 12 with reduction rate of 43.4% and 44.1%. In addition, the serum brought significant improvement on elasticity at 4 mini-sites (forehead, underneath eye, cheek, nasolabial) on the face with 39.1%, 17.2%, 8.8%, 10.1% improvement rates at week 12, respectively.

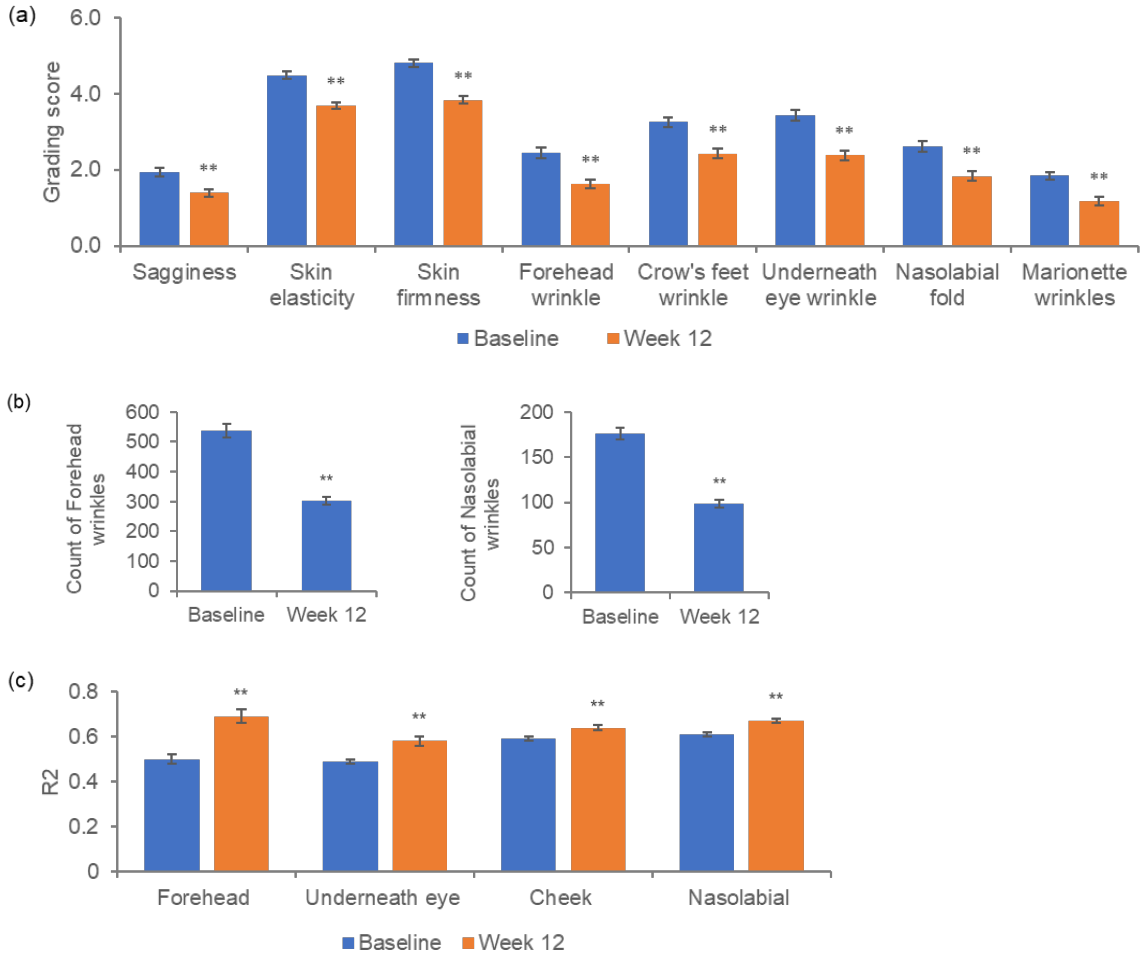

Figure 4. Anti-aging efficacy clinical and instrumental evaluation result in China. (a) Clinical grading score of sagginess, elasticity, firmness, forehead wrinkle, crow's feet wrinkle, underneath eye wrinkle, nasolabial fold, marionette wrinkle before and after 12 weeks of serum application. (b) The decrease on count of forehead wrinkles and nasolabial wrinkles after 12 weeks application. (c) Improvement on skin elasticity R2 value at week 12. **p<0.05 versus baseline.

3.5. The study was finally completed in a group of 76 women with Fitzpatrick skin type I-VI. Compared with clinical grading baseline, the serum delivered significant ($p < 0.05$) efficacy on improving skin quality related attributes: firmness, elasticity, sagginess, and skin wrinkle related attributes: marionette line, forehead wrinkle, nasolabial fold, crow's feet wrinkle, underneath eye wrinkle after 12 weeks repeated applications.

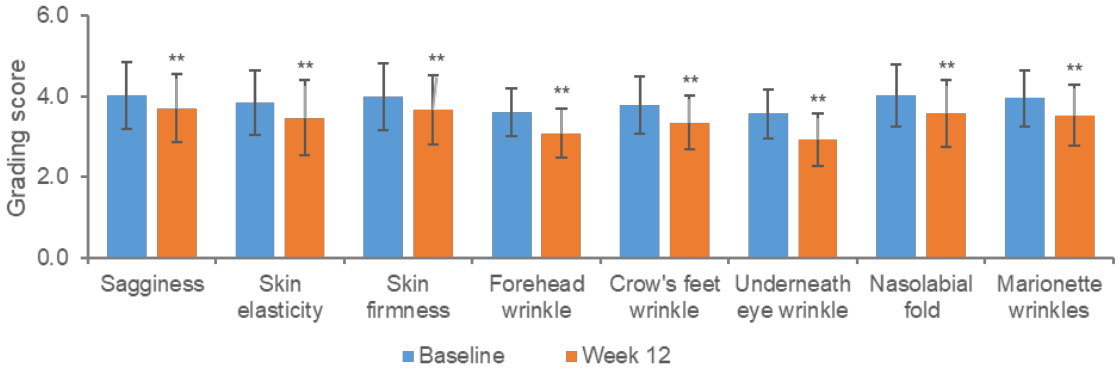

Figure 5. Anti-aging efficacy clinical evaluation result in Canada. Clinical grading score of sagginess, elasticity, firmness, forehead wrinkle, crow's feet wrinkle, underneath eye wrinkle, nasolabial fold, marionette wrinkle before and after 12 weeks of serum application.

4. Discussion

Previously, AGEs accumulation was evaluated by skin biopsies, which are not appropriate for routine clinical study due to the invasiveness. Recently, commercially available, non-invasive device AGE READER was validated to measure AGEs level by using SAF value and measurement area is usually on the forearm [10]. However, the face is more relevant to the actual scenario of cosmetics application, and previous research has shown a strong correlation between the level of AGEs on the face and arm to support facial AGEs level as a measurement parameter [2]. Therefore, this study assessed facial AGEs to evaluate the anti-glycation performance on serum products. Meanwhile, inner upper arms are considered as the skin area with no sun exposure, product was applied on one side according to randomization table while another side was designed for blank control.

In the skin, UV exposure may precipitate the formation of AGEs and exacerbate skin glycation [4, 7]. Crisan et al. found that AGE accumulation, e.g. CML, is higher in sun-exposed skin compared to sun unexposed skin [12]. However, there are several studies that indicated that the average SAF value on the sun-exposed skin area was lower than sun unexposed skin region. Larsson et al. argued that sun exposure can cause an increased absorption of fluorescence light that leads to lower SAF value [11]. In our study, the baseline of average SAF value on the facial skin was lower than upper arm skin area, suggested AGEs content might be different

among different body sites. However, the serum which contains 3 flavonoids significantly reduced SAF value both on facial and upper arm region.

Previous studies have shown that C-xyloside stimulated increased glycosaminoglycan synthesis in human dermis and improved the ultrastructure of the DEJ. Clinical studies have demonstrated that C-xyloside can significantly improve facial wrinkles, radiance, evenness, and firmness [3]. In our 2 clinical studies, serum contains 3 flavonoids and C-xyloside showed significant improvement on wrinkles with multiple ethnicities. Hence, the combination of anti-glycation mechanism and ECM & DEJ remodeling is an effective anti-aging strategy.

5. Conclusion

For the first time, we demonstrated Cassia Alata extracts synergistically increased the anti-glycation efficacy of fruit flavonoids. It provided sufficient evidence that combined mechanisms of anti-glycation and ECM & DEJ remodeling can significantly reduce skin glycation and address aging-related issues such as elasticity, sagging, wrinkle issues. Our research provides insights that might broaden the potential applications of cosmetics, such as photoaging prevention and scalp care.

Conflict of Interest Statement

NONE

References

[1] Farrar MD. Advanced glycation end products in skin ageing and photoageing: what are the implications for epidermal function? Exp Dermatol. 2016 Dec;25(12):947-948.

[2] Zhang Q, Cheng D, Wang F. Facial assessment methods for inhibiting glycation and aging effects and the correlation between glycation and aging parameters. Sci Rep. 2025 Jan 14;15(1):1935.

[3] Zang S, Chen J, Chevalier C, Zhang J, Li S, Wang H, Li J, Chen Y, Xu H, Sheng L, Zhang Z, Qiu J. Holistic investigation of the anti-wrinkle and repair efficacy of a facial cream enriched with C-xyloside. J Cosmet Dermatol. 2024 Dec;23(12):4017-4028.

[4] Zheng W, Li H, Go Y, Chan XHF, Huang Q, Wu J. Research Advances on the Damage Mechanism of Skin Glycation and Related Inhibitors. Nutrients. 2022 Nov 1;14(21):4588.

[5] Atzeni IM, van de Zande SC, Westra J, Zwerver J, Smit AJ, Mulder DJ. The AGE Reader: A non-invasive method to assess long-term tissue damage. Methods. 2022 Jul;203:533-541.

[6] Chen CY, Zhang JQ, Li L, Guo MM, He YF, Dong YM, Meng H, Yi F. Advanced Glycation End Products in the Skin: Molecular Mechanisms, Methods of Measurement, and Inhibitory Pathways. Front Med (Lausanne). 2022 May 11;9:837222.

[7] Gkogkolou P, Böhm M. Advanced glycation end products: Key players in skin aging? Dermatoendocrinol. 2012 Jul 1;4(3):259-70.

[8] Martinovic D, Tokic D, Usljebrka M, Lupi-Ferandin S, Cigic L, Vanjaka Rogosic L, Ercegovic S, Kontic M, Kumrić M, Rusic D, Vilovic M, Leskur M, Bozic J. The Association between the Level of Advanced Glycation End Products and Objective Skin Quality Parameters. Life (Basel). 2023 Jan 17;13(2):256.

[9] Abbas, G.; Al-Harrasi, A.S.; Hussain, H.; Hussain, J.; Rashid, R.; Choudhary, M.I. Antiglycation Therapy: Discovery of Promising Antiglycation Agents for the Management of Diabetic Complications. Pharm. Biol. 2016, 54, 198–206.

[10] Adl Amini D, Moser M, Chiapparelli E, Oezel L, Zhu J, Okano I, Shue J, Sama AA, Cammisa FP, Girardi FP, Hughes AP. A Prospective Analysis of Skin and Fingertip Advanced Glycation End-Product Devices in Healthy Volunteers. J Clin Med. 2022 Aug 12;11(16):4709.

[11] Larsson M, Favilla R, Strömberg T. Assessment of advanced glycated end product accumulation in skin using auto fluorescence multispectral imaging. Comput Biol Med. 2017 Jun 1;85:106-111.

[12] Crisan M, Taulescu M, Crisan D, Cosgarea R, Parvu A, Cãtoi C, Drugan T. Expression of advanced glycation end-products on sun-exposed and non-exposed cutaneous sites during the ageing process in humans. PLoS One. 2013 Oct 7;8(10):e75003.