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"A PASSIFLORA EDULIS EXTRACT WITH A POTENT STIMULATION OF VITAMIN D3 SYNTHESIS IN SKIN EPIDERMIS: IN SILICO AND IN VITRO"

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1. Introduction

Vitamin D3 plays an important role in the body. The most important source of vitamin D3 is sun exposure because of biochemical conversion of 7-dehydrocholesterol into pre-vitamin D3. However, hypovitaminosis D3 is prevalent in developed and developing countries among a population of all ages. The analysis shows that the global prevalence of vitamin D deficiency was 15.7% between 2000 and 2022 [1]. Lack of vitamin D3 leads to premature dryness, hyperpigmentation, loss of elasticity, formation of wrinkles especially in skin and different health implications. Moreover, vitamin D deficiency is associated with the increase of symptoms of mood disorders such as depression and anxiety, which can significantly impact well-being [2]. Vitamin D synthesis is possible without sunlight in plants, lichens, even some mammals, but in humans, vitamin D synthesis is possible only in the presence of UVB, which means exposure to the sun is a prerequisite [3]. Thus, the aim of this research was to investigate *Passiflora edulis* extract for stimulation vitamin D3 synthesis in skin with and without UVB radiation to provide a normal balance.

2. Materials and Methods

In silico research was conducted through Autodock 4.2 version. The native protein ligand was initially implemented in the molecular docking process to verify procedural consistency, wherein the root mean square deviation (RMSD) was confirmed to be less than 2 Å. Grids

coordinates were established at (X, Y, Z) 43.353, -17.01, 101.091 (Lanosterol oxidase); -13.248, -5.581, 24.162 (vitamin D3 receptor) and a grid box of dimensions 40 × 40 × 40 was configured. Throughout the docking procedure, ligand flexibility was permitted while macromolecular rigidity was maintained. Lanosterol oxidase (PDB ID: 3LD6) and vitamin D3 receptor (PDB ID: 4ITF) was subjected to docking with 10 molecules and was examined utilizing AutoDock 4.2 version.

Cell viability was determined by a colorimetric method using MTT dye (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; Sigma, St. Lous, MO). For the assay the product was prepared in culture medium with 3% Tween 20 (Sigma) and added to the 96 well plate at a serial dilution in the range of 100.00 to 0.003 mg/mL using the dilution factor of 3.16. The human skin keratinocytes culture was incubated for a period of 48 hours. MTT was then added to the culture at a concentration of 5mg/mL (30µL / well) and incubated for additional 4 hours. The contents of the well were removed and 100 µl of isopropanol was added for the purpose of solubilizing the formazan crystals formed by viable cells. The absorbance of each well was determined at 570 nm in Multiskan GO monochromator (Thermo Scientific, Finland).

Primary human keratinocytes were seeded in 75 cm^2 flasks (Corning, USA), cultured and expanded in 37°C incubator in the presence of 5% CO2. Upon reaching confluence, keratinocytes were seeded in 6 wells for further quantification of vitamin D3.

Concentrations of Vitamin D3 were measured in the supernatant by ELISA method, accordingly to the protocol on the datasheet provided by the supplier, using a commercially available kit (Elabscience, USA). The 450 nm absorbance reading was performed in Multiskan GO monochromator (Thermo Fisher Scientific, Finland).

To evaluate the data obtained, the ANOVA test was used, which also allowed measuring the variation in results, comparing data between groups. The Bonferroni post-test was then applied, which reinforced and made the result presented in the ANOVA test even more precise.

3. Results

Through *in silico* analysis to predict the affinity to lanosterol oxidase and vitamin D3 receptor by AutoDock, chlorogenic acid as a part of *Passiflora edulis* extract was chosen for a stimulation of vitamin D3 synthesis in skin. An appreciable number of flowering plants, specially within the *Solanaceae* family, have been shown to contain vitamin D3 and its hydroxylated derivatives, including 1a,25(OH)2-vitamin D3 [1a,25(OH)D3], a pluripotent hormone in animals. These secosteroids have also been detected in members of the *Cucurbitaceae*, *Fabaceae* and *Poaceae* families [4]. *Passiflora edulis* was not considered as a plant containing

vitamin D3 and its hydroxylated derivatives, thus it is necessary to investigate the effects of Passiflora edulis to verify the results of in silico analysis.

Firstly, the determination of non-cytotoxic concentration for further evaluation was performed by a colorimetric method using MTT dye. Secondly, human keratinocytes were incubated with standardized Passiflora edulis extract at 3 non-cytotoxic concentrations of 10.01, 3.17 and 1.00 mg/mL in 37°C for 72 hours. Some of keratinocytes were also exposed to 50 mJ/cm2 of UVB to compare the irradiated and non-irradiated cultures treated with the extract. Then, the supernatant was collected for quantification of the vitamin D3 and analyzed using ELISA assay. It was established that *Passiflora edulis* extract showed a good toxicological profile. The extract promoted the production of vitamin D3 in skin epidermal cells (Figure 1). Moreover, the *Passiflora edulis* extract at concentrations of 10.01, 3.17, and 1.00 mg/mL increased of vitamin D3 amounts up to 274.04%, 151.22%, and 125.86% respectively, compared to the basal control without UVB influence ($p<0.01$).

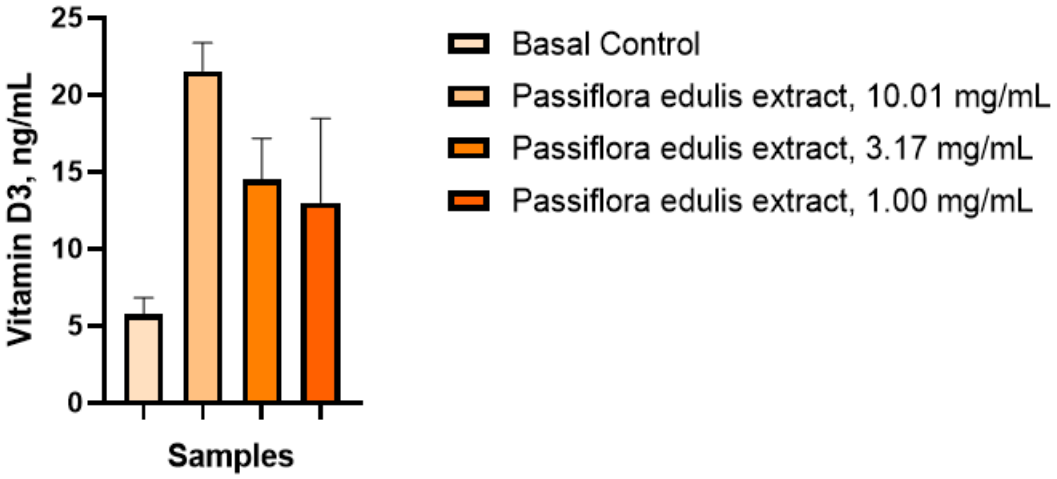

Figure 1. Determination of Vitamin D3 in skin epidermal cells after incubation with Passiflora edulis extract without UVB radiation.

As anticipated, exposure to UVB radiation exhibited significant increase in vitamin D3 up to 146% compared to the basal control ($p<0.01$). Nevertheless, the *Passiflora edulis* extract at concentrations of 10.01 and 3.17 mg/mL under UVB radiation demonstrated noteworthy effects (Figure 2), significantly increasing vitamin D3 up to 61.41% and 45.82% respectively, compared to the UVB positive control ($p<0.05$).

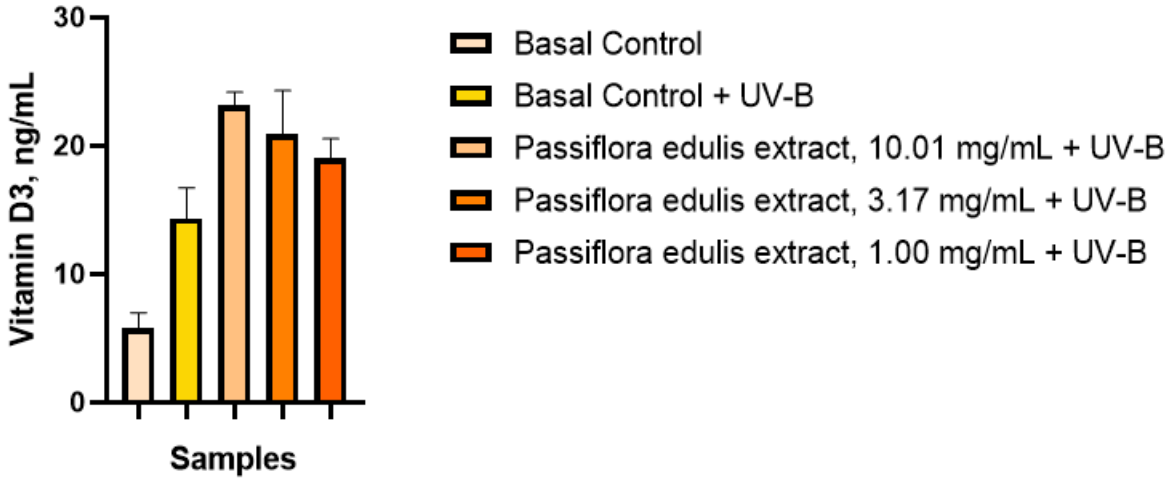

Figure 2. Determination of Vitamin D3 in skin epidermal cells after incubation with Passiflora edulis extract with UV-B radiation.

The novel *Passiflora edulis* extract worked as a booster of vitamin D3 synthesis for anti-ageing effect. The obtained results confirmed that the *Passiflora edulis* extract enriched chlorogenic acid has a beneficial effect to stimulate the vitamin D3 synthesis in skin epidermis under or without UVB radiation. Nevertheless, additional studies of stability in formulations, dermatological tolerance in the cosmeceutical products, and clinical research of vitamin D3 level are needed to fully confirm the beneficial effects.

4. Discussion

In this study, it was demonstrated that the *Passiflora edulis* extract has the capacity to modulate vitamin D3 synthesis in skin keratinocytes. This effect has not been previously reported in the literature, as neither vitamin D3 nor its precursors have been documented in passion fruit. The most significant finding is the *Passiflora edulis* extract's ability to exert this effect even in the absence of ultraviolet (UVB) exposure, while also synergistically enhancing UV-induced vitamin D3 production. Subsequent research and product development based on this technology could lead to new approaches for addressing the global prevalence of vitamin D3 deficiency in skin, particularly in regions with limited sunlight.

5. Conclusion

The novel *Passiflora edulis* extract worked as a booster of vitamin D3 synthesis for anti-aging effect. The obtained results confirmed that the *Passiflora edulis* extract enriched chlorogenic acid has a beneficial effect to stimulate the vitamin D3 synthesis in skin epidermis under or without UVB radiation. Nevertheless, additional studies of stability in formulations, dermatological tolerance in the cosmeceutical products, and clinical research of vitamin D3 level are needed to fully confirm the beneficial effects.

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