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Molecular Modelling and In Vitro Research of New Substances for the Targeted Stimulation of AQP3 in Skin †

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Abstract: Skin dryness and xerosis are the most common clinical manifestations of different dermatological diseases. Meanwhile, it was established that expression of aquaporines 3 (AQP3) is related to the pathogenesis of atopic dermatitis, psoriasis, eczema, and vitiligo. Thus, our study was focused on a search of new molecules and investigation their biological activity to accelerate expression of AQP3 in skin epidermis. Aloin from *Aloe barbadensis* leaf extract and trimethylglycine were chosen as new potential candidates using DiffDock computational modelling. These natural molecules demonstrated a good affinity towards the active site of AQP3 with an estimated docking score from −6.2 kcal/mol to −7.7 kcal/mol. Phyto4Health modelling predicted the anti-psoriatic, anti-inflammatory, and immunosuppressant activities useful for the treatment of skin atopic diseases. Furthermore, it was shown that combination of *Aloe barbadensis* leaf extract and trimethylglycine in a mass ratio of 1:1 revealed a clear synergetic effect to increase AQP3 amount up to 2 times. Thus, the combination of *Aloe barbadensis* extract standardized for aloin and trimethylglycine has a promising potential in the drug development and treatment of dryness.

Keywords: aloe vera; trimethylglycine; aquaporines; skin hydration; synergy; docking

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1. Introduction

Skin performs its functions only in case its barrier layers is not damaged, and sufficient transepidermal water flow is provided. It was established that skin xerosis is the most common clinical manifestations of different dermatological diseases, such as atopic dermatitis, eczema, psoriasis, and vitiligo [1]. The prevalence of dry skin by age groups in Europe is occurred in up to 56% of elderly people around the world and up to 75% of all population [1]. The results of research showed that the clinically recommended emollients targeted on skin hydration and recovery of epidermal lipid barrier aren't sufficient in the treatment of skin atopic diseases and may make skin dryer [2].

Skin performs its functions only in case its barrier layers is not damaged, and sufficient transepidermal water flow is provided. Water is vitally important for natural functioning and healthy skin look, as skin is the largest external body organ with three separate layers: epidermis, dermis, and hypodermis. Skin hydration is the result of interaction of the three basic mechanisms: the corneal layer and its barrier role in relation to water loss, natural moisturizing factor (NMF), including several hygroscopic molecules to maintain corneocytes hydration, and water transportation channels, such as aquaporines of 3 types (AQP3). AQP3 provide a transport of water, glycerol and molecules of natural

moisturizing factor increasing the skin hydration, proliferation of keratinocytes and wound healing [3]. AQP3 is an essential element for sufficient skin hydration [4].

It is found out that skin aging is related to decrease of moisture in skin, increased TEWL parameter and decreased amount of aquaporins type 3 (AQP3) in epidermal cells [5]. Meanwhile, it was established that expression of aquaporines 3 (AQP3) is related to the pathogenesis of atopic dermatitis [6], psoriasis [7], chronic skin irritation [8], and vitiligo [9]. In the pathogenesis of psoriasis, the decrease of AQP3 amount due to excessive immunity reaction is specified [10].

Stimulation of AQP3 gene expression and protein translocation can be a potential mechanism to prevent and treat these dermatological diseases. Due to the important role of AQP3 in skin and the necessity of regulation of AQP3 amount in epidermal skin, the search for innovative plant-based combination for long-term skin moisturizing, keratinocytes proliferation and skin barrier function maintenance remains urgent [11].

Plant-based substances, such as essential oils or plant extracts, have a comprehensive compositions, multiple properties, different activities, and low irritancy potential [12]. Extracts of various plants are irreplaceable components in dermatological products due to their anti-inflammatory, antioxidating, moisturizing and protective effects [13]. It was discovered by the authors, the most promising substance for the treatment of skin xerosis are *Aloe barbadensis* leaf extract due to richness of biologically active molecules [14] and [15].

2. Materials and Methods

2.1. Chemicals and Materials

Aloe barbadensis leaf extract (CAS 85507-69-3) standardized in aloin content, and trimethylglycine (CAS 107-43-7), were purchased from Sigma-Aldrich (Sigma Chemical Co., Ltd., St. Louis, MO, USA). Glyceryl glucoside Hydagen® Aquaporin was purchased from BASF (BASF Personal Care and Nutrition GmbH, Monheim, Germany). All chemicals used in the in vitro research were of analytical grade. Keratinocytes HaCaT and Aquaporin 3 ELISA kit purchased from MatTek (MatTek Europe, Bratislava, Slovakia).

2.2. Ligand and Target Preparation

The AQP3 protein (PDB ID: 3LLQ) (Figure 1) was used to fit the three-dimensional structure of the AQP3. The protein was obtained in the.pdb format from the Protein Data Bank [16]. Using AutoDock version 4.2, the protein model was prepared by eliminating water molecules, cutting out superfluous chains, and adding polar hydrogen and charges. After processing, the protein structure was saved in the.pdb and pdbqt formats for additional in silico study.

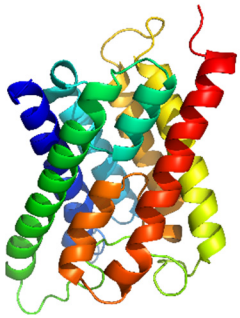


Figure 1. 3D structure of AQP3 from Protein Data Bank.

2.3. Molecular Docking of Phytochemicals with AQP3

A personal computer (PC) with an Intel Core i7-12700U CPU running at 2.3 GHz and 16 GB of RAM was the tool used for this purpose. Windows 11, 64-bit OS, was the operating system in use. Firstly, the native protein ligand was used in the molecular docking process to confirm that the procedure was consistent, and the root mean square deviation (RMSD) was less than 2 Å. The coordinates of the grid are (X, Y, Z) 28.73, 58.834, 63.068 and the grid box is 40 × 40 × 40. The ligand was flexible, and the macromolecule remained rigid during the docking process. AQP3 (PDB ID: 3LLQ) was docked with 10 molecules and explored using AutoDock version 4.2. The molecular docking was carried out by modifying the parameter of the genetic algorithm (GA), using ten runs of the criterion of Lamarckian GA.

2.4. Drug-Likeness Activity

The drug-likeness analysis was carried out with Phyto4Health (https://www.way2drug.com/p4h/ (accessed on)) to predict biological activities for natural molecules of plant origin [17]. This database contains information about more than 9000 phytoconstituents from different medical plants and herbs. All structures of phytocompounds are presented in InChI, InChi Key Canonical SMILES formats. This in silico program is suitable to predict the affinity of ligands to target, biological activity with approximate PASS effects and compare the physicochemical properties of molecules, such as hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), number of rotatable bonds (RTB), polar surface area (PSA) and octanol-water partition coefficient (AlogP).

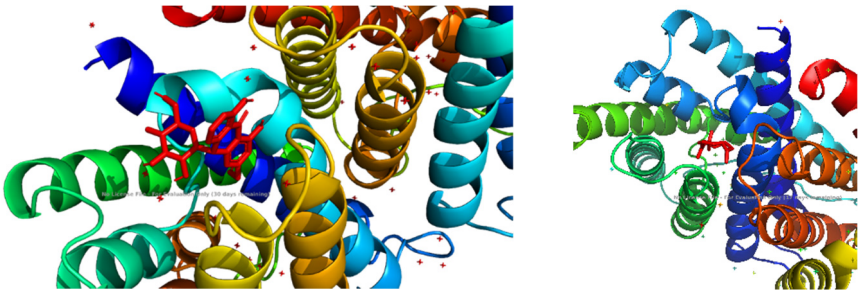
2.5. In Vitro Research of AQP3 Amount in Epidermis Cells

To determine the amount of AQP3 in epidermal cells, a commercially available kit with the sandwich-type enzyme-linked immunoassay was used (MatTek Europe, Bratislava, Slovakia). Cells of HaCaT line were seeded in 96-well plates (96 Well EDGE Cell Culture Plates, Nest Scientific Biotechnology, Wuxi, China) at a concentration of 1×10^5 cells per well. On the next day, the cell media was removed and replaced with fresh DMEM media + 5% FBS 50 μL to maintain cell growth. Then 50 μL of samples of the composition were added, and cultivation was performed for 24 h. After incubation, the cell supernatant was collected, and the amount of AQP3 in skin keratinocytes was determined by ELISA assay. The standards and samples were added to the corresponding microplate wells with specific biotin-conjugated AQP3 antibodies. Horseradish peroxidase-conjugated avidin was then added to each microplate well and incubated. After the addition of TMB substrate solution to each microplate well, the enzyme-substrate reaction was stopped by adding a sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 ± 10 nm. There are negative control (purified water) and positive control (glyceryl glucoside).

3. Results and Discussion

3.1. Molecular Docking with AQP3

Using AutoDock computational modelling to predict affinity for skin AQP3, aloin from *Aloe barbadensis* leaf extract (Figure 2b) and trimethylglycine (Figure 2a) were chosen as new potential candidates. These natural molecules demonstrated a good affinity towards the active site of AQP3 with an estimated docking score from −6.2 kcal/mol to −7.7 kcal/mol. To improve the affinity and stabilize the structure of AQP3, trimethylglycine as natural osmolyte could be useful. The glyceryl glucoside as a positive control had a moderate affinity with an estimated docking score −4.0 kcal/mol.



(**a**) (**b**)

Figure 2. (**a**) Detailed docking of AQP3 with aloin from *Aloe Barbadensis* leaf extract; (**b**) Detailed docking of AQP3 with trimethylglycine.

3.2. Drug-Likeness Activity

Thorough Phyto4Health modelling to predict the pharmacological properties, it was established that aloin from *Aloe barbadensis* leaf extract and trimethylglycine could have anti-psoriatic, antioxidant, anti-inflammatory, and immunosuppressant activities useful for the treatment of skin atopic diseases [17]. The main biological activities useful for skin disorders and skin hydration level are presented in Table 1.

Table 1. Biological activities of Aloin and Trimethylglycine using Phyto4Health database.

Compound	PASS Activities Type	Pa Value
Aloin	Antioxidant	0.676
	Anti-inflammatory	0.674
	Immunosuppressant	0.524
Trimethylglycine	Antieczematic atopic	0.806
	Anti-psoriatic	0.570
	Antioxidant	0.259

3.3. In Vitro Research of AQP3 Amount in Epidermis Cells

It was revealed that the addition of the *Aloe barbadensis* leaf extract standardized for aloin, trimethylglycine, and the combination of these both phytochemicals in a 1:1 mass ratio to the epidermal cells increase the AQP3 amount (Table 2). The combination of *Aloe barbadensis* leaf extract and trimethylglycine increased the amount of AQP3 to 12.21 ± 0.91 ng/mL compared to the negative control—5.58 ± 0.24 ng/mL. The glyceryl glucoside in a mass concentration of 1% induced the amount of AQP3 in epidermal cells, but this influence was less than that of the novel plant-based combination.

Table 2. The effects of compounds on AQP3 amount.

Compound	AQP3, ng/mL	Changes Compared with the Negative Control, %
Negative control (purified water)	5.58 ± 0.24	-
Aloe barbadensis leaf extract with aloin, 1.0 weight %	6.73 ± 0.69 *	+20.61% *
Trimethylglycine, 1.0 weight %	6.58 ± 1.08	+17.92%
Aloe barbadensis leaf extract and trimethylglycine in a 1:1 mass ratio, 1.0 weight %	12.21 ± 0.91 **	+118.82% ***
Glyceryl glucoside, 1.0 weight %	6.89 ± 082 *	+23.48% *

Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Conclusions

The novel plant-based combination with the investigated AQP3 targeted activity for the treatment of skin xerosis was developed. The combination of *Aloe barbadensis* leaf extract and trimethylglycine in a 1:1 mass ratio had a significant increase of AQP3 amount in epidermal cells, compared to the negative and positive control. Thus, the investigated plant-based substance has a promising potential for the treatment of skin disorders. However, additional research of toxicity, dermal tolerance, allergic potential and clinical efficiency are needed to confirm this activity and beneficial effect on skin.

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