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The novel combination based on *Aloe vera* extract and trimethylglycine for targeted AQP3 stimulation and skin hydration

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

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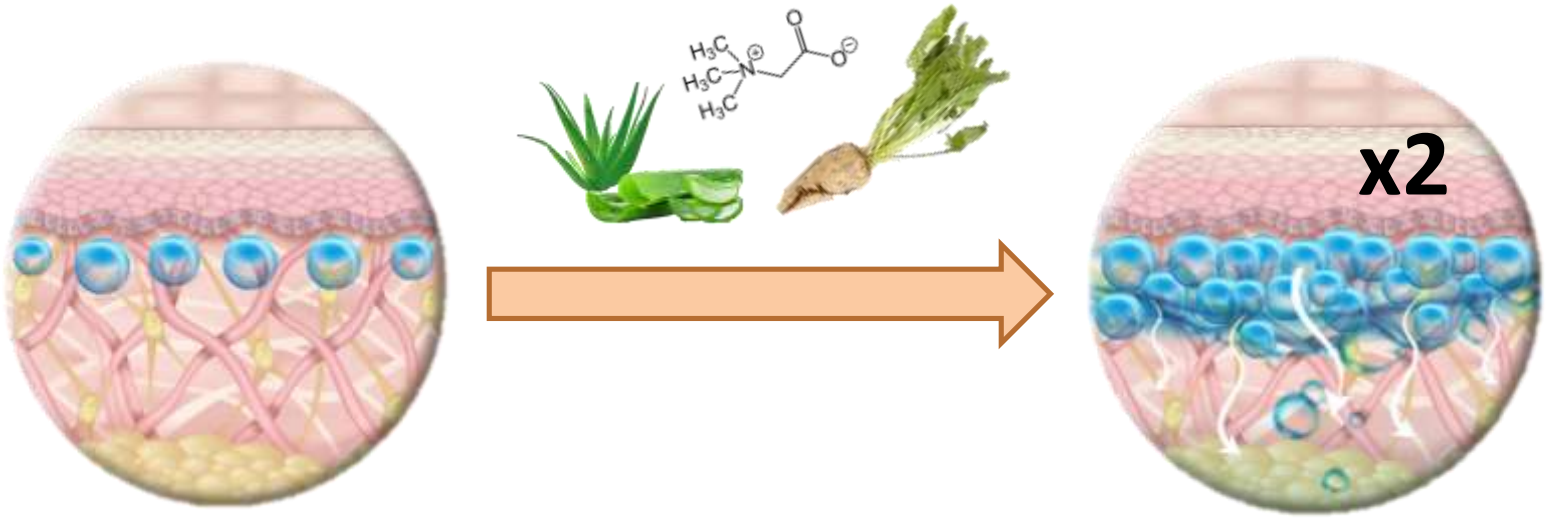
SKYLAB

LOMONOSOV MOSCOW STATE UNIVERSITY

The novel combination based on *Aloe vera* extract and trimethylglycine for targeted AQP3 stimulation and skin hydration



Standard AQP3 amount in skin epidermis

AQP3 amount in skin epidermis after use of the novel combination

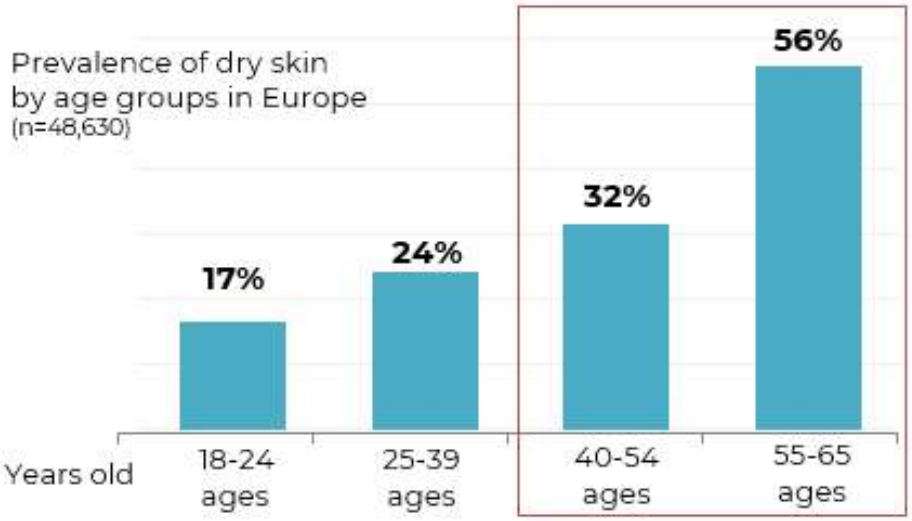
Abstract: The aquaporins 3 (AQP3) are tetrameric membrane-bound channels that facilitate transport of water and other small solutes across skin cell membranes. The recent findings revealed that AQP3 are involved in the progression of skin disorders, such as atopic dermatitis, psoriasis, eczema, vitiligo, and xerosis. Research of novel combination of plant molecules that could increase the expression of this protein for skin hydration is currently ongoing. Through DiffDock computational modelling to predict affinity for AQP3 and the biological activity, Aloe vera extract and trimethylglycine were chosen. Thus, our science work was focused on the development of a novel combination based on Aloe vera extract and trimethylglycine and evaluation of targeted AQP3 regulation in skin keratinocytes in the presence of this combination. Firstly, the cytotoxicity assay of selected substances was performed with MTT indicator on HaCaT cells. Secondly, the substances' ability to increase amount of AQP3 was evaluated in the keratinocytes' cell culture with ELISA immunoassay. According to the results obtained, the novel combination based on Aloe vera extract and trimethylglycine in a mass ratio of 1:1 had a good cytotoxicity profile, with an EC70 value of 11.95%. Moreover, it was shown that the combination had a clear synergetic activity and significantly increased amount of epidermal AQP3 up to 219%, compared to that of negative control ($p<0.001$). Thus, the novel combination of plant molecules has a promising potential for the development of dermatological drugs and the treatment of skin disorders related to the low skin hydration.

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

Skin dryness and xerosis: overview

GLOBAL PREVALENCE OF SKIN XEROSIS

from **30** to **75%**
of population from different countries
has skin xerosis in the mild or moderate form



MAIN MANIFESTATIONS OF SKIN XEROSIS

- Skin dryness
- Visible desquamation
- Skin sensitivity
- Skin itching
- Mild skin erythema

GLOBAL ANALYTICS OF MOISTURIZERS IN DIFFERENT COUNTRIES

20% of people aged 16-24 select only products for skin dryness

26% of people aged 18-49 select only products for skin dryness

59% of people aged 18-49 select only products for skin dryness

Analytic source: Mintel Analytics (KuRunData/Mintel Lightspeed/Mintel) and (Cipher/Mintel).
Paul C, Maumus-Robert S, Mazereeuw-Hautier J, et al. Prevalence and risk factors for xerosis: a cross-sectional epidemiological study in primary care. Dermatology. 2011;223(3):260-5.
Polat M, Yalcin B, Calikkan D, Alli N. Complete dermatological examination in the elderly: an exploratory study from an outpatient clinic in Turkey. Gerontology 2009;55:58-63.

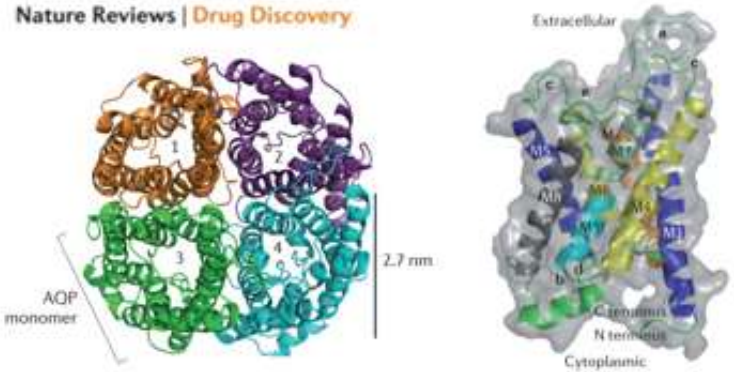
Role of aquaporines 3 in skin health and xerosis

The Nobel Prize in Chemistry 2003

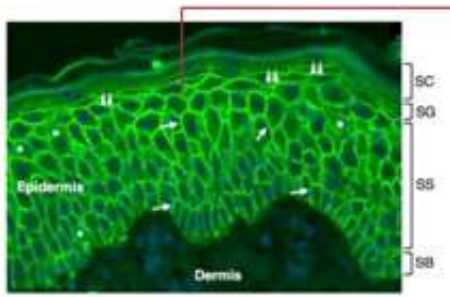
Peter Agre, Johns Hopkins University
Roderick MacKinnon, Rockefeller University



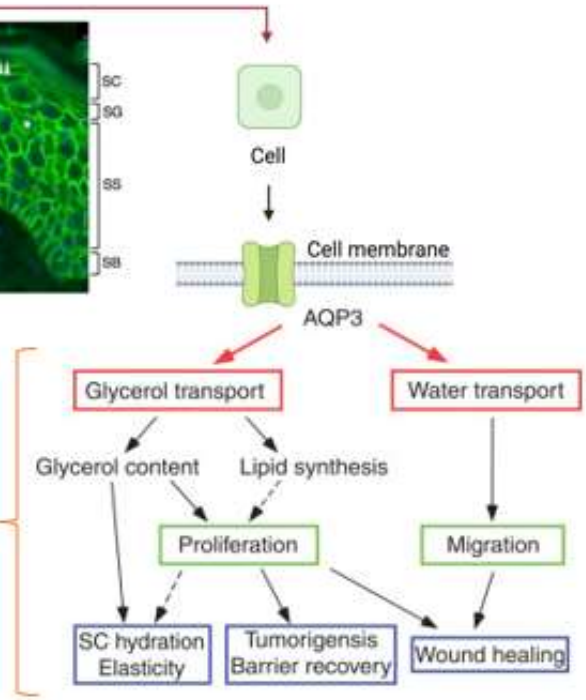
The structure of aquaporines 3 (AQP3)



AQP3 provides skin hydration, skin barrier repair, and wound healing



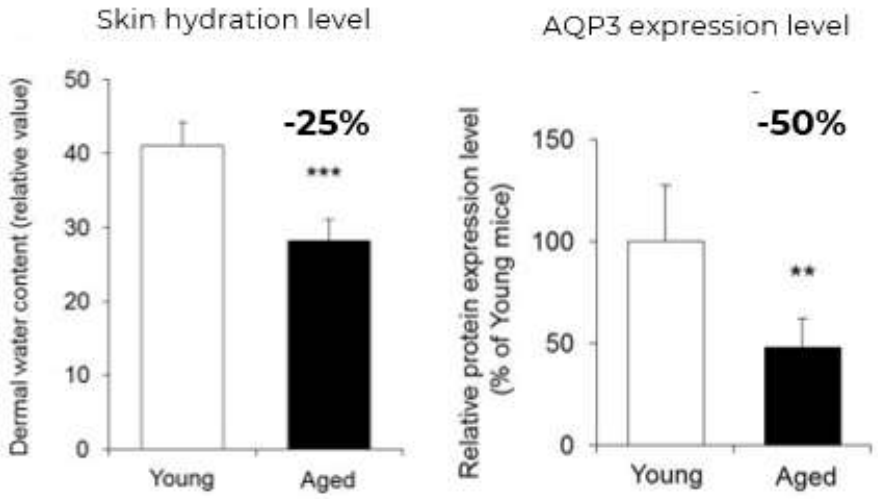
- Skin ageing
- Oxidative stress
- Inflammation
- UVB radiation
- Skin pH level
- Heavy metals



Sources: https://www.nature.com/articles/nrd4226; Tricarico, P.M.; Mentino, D.; De Marco, A.; Del Vecchio, C.; Cairo, S.; Cazzato, C.; Foti, C.; Crovella, S.; Calamita, C. Aquaporins Are One of the Critical Factors in the Disruption of the Skin Barrier in Inflammatory Skin Diseases. Int. J. Mol. Sci. 2022, 23, 4020.

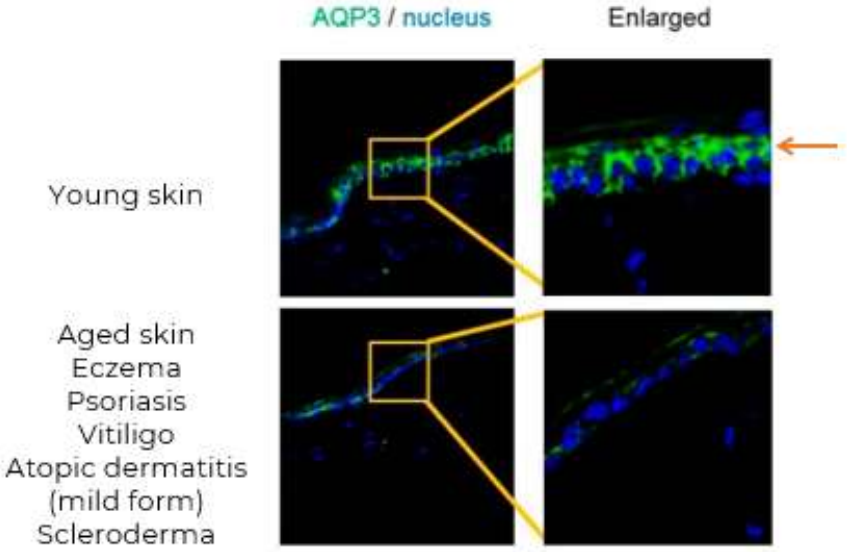
The expression level of AQPs is one of the causes of dermatological diseases

Ageing significantly influences on the expression level of AQP3 in the skin



the data represent the means ± SDs for five mice.
Student's t-test: ** $p < 0.01$ vs. Young mice (100%).
Young mice = 3 months; Aged mice = 20 months.

AQP3 are one of the critical factors in the disruption of the skin barrier in inflammatory skin diseases



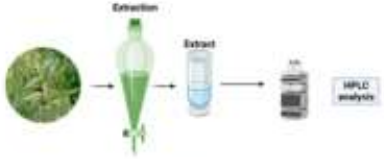
Sources: Ikarashi N, Kon R, Kaneko M, Mizukami N, Kusunoki Y, Sugiyama K. Relationship between Aging-Related Skin Dryness and Aquaporins. Int J Mol Sci. 2017 Jul 18;18(7):1559.
Ballag WB, Aitkens L, White J, Hyndman KA. Aquaporin-3 in the epidermis: more than skin deep. Am J Physiol Cell Physiol. 2020 Jun 1;318(6):C1144-C1153.

Aim of study and first tasks for scientific group

The research of a novel plant-based combination
and biological evaluation its activity for AQP3 regulation in skin epidermis

Virtual screening
AutoDock docking

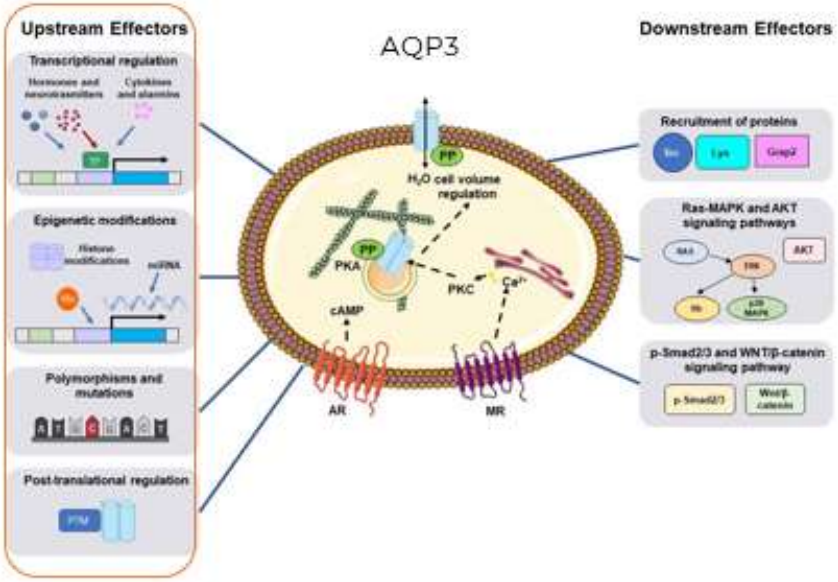
Search, preparation and
analysis of phytochemicals



Cell viability assay
on HaCaT epidermal cells

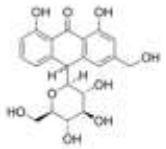
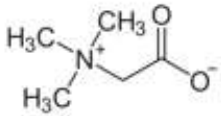
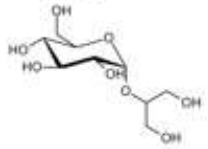
In vitro study of AQP3
quantification in skin epidermis

The virtual screening was performed using AutoDock and Phyto4Health



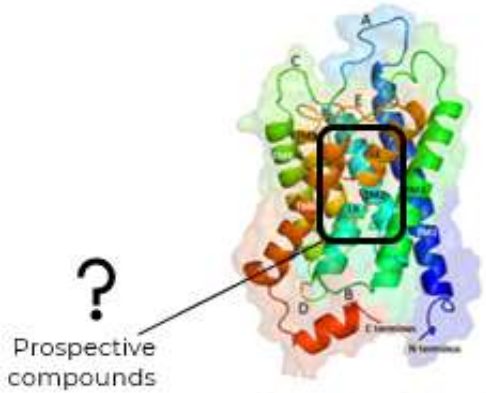
The mechanisms through which the AQP3 expression is regulated in the epidermis are just starting to be understood...

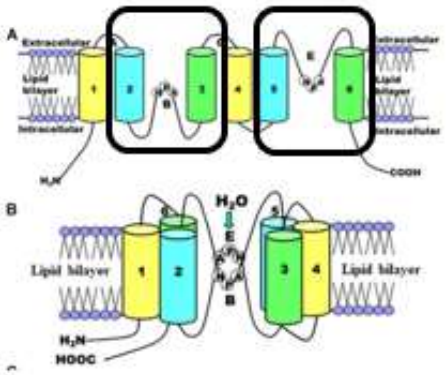
The chemicals for increase of AQP3 expression

Aloin	Trimethylglycine	Glyceryl glucoside
		
AQP3 gene expression (PPARγ, HDAC3...)	Post translation modification (methylation etc.)	AQP3 gene expression (?)

Phytochemicals were chosen via AutoDock AI

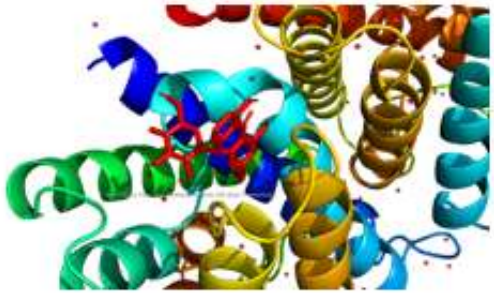
Aquaporin 3 in skin epidermal cells





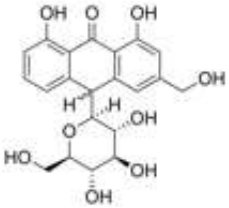
Source: Azad AK, Rashan T, Ahmed J, Hakim A, Emon TH, Chowdhury PA. Human Aquaporins: Functional Diversity and Potential Roles in Infectious and Non-Infectious Diseases. Front Genet. 2021 Mar 16;12:654865.

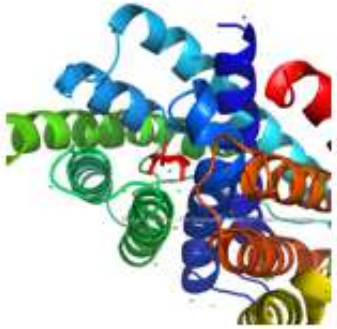
Docking of chemicals with AQP3



Aloin

TM2 and TM3 loops
TM5 and TM6 loops
-7.7 kkal/mol

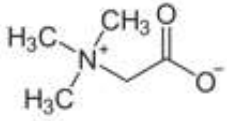


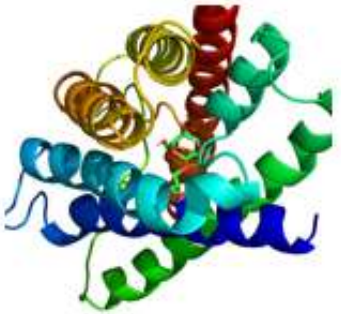


Trimethylglycine

TM2 and TM3 loops

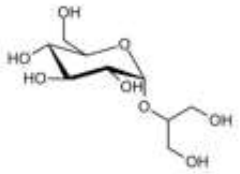
-2.7 kkal/mol





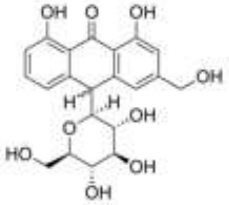
Glyceryl glucoside
(active control)
TM2, TM3 and LB loops

-4.0 kkal/mol

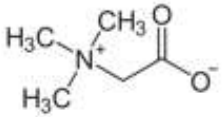


Chosen phytochemicals and analysis one of them

Substances



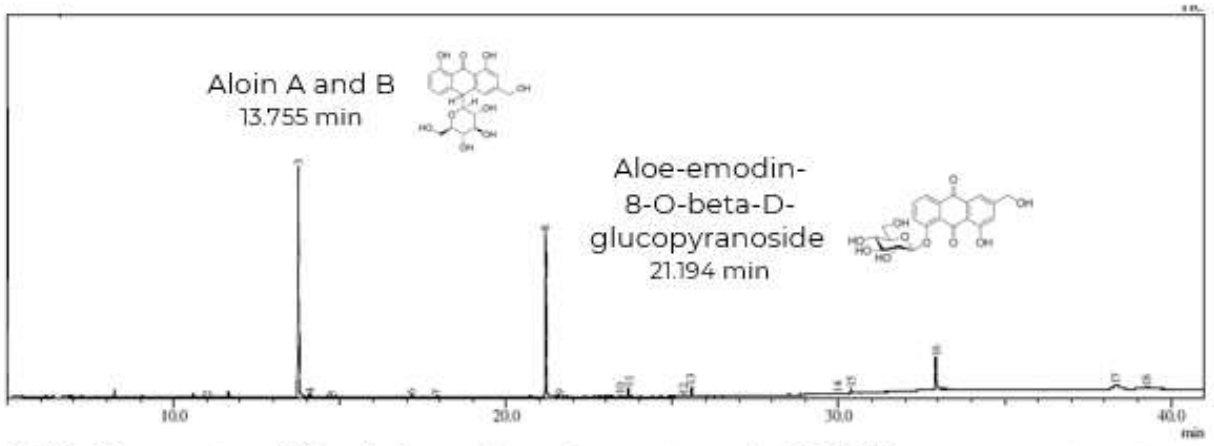
Aloe barbadensis leaf extract standardized for aloin A and B
extraction by 50% methanol in water (v/v)



Trimethylglycine from *Beta vulgaris* root extract
(substance with 99.9% purity)

HPLC-MS analysis of *Aloe barbadensis* leaf extract

18 identified compounds in *Aloe barbadensis* leaf extract
Aloin A and B (39.95%), Aloe-emodin-8-O-beta-D-glucopyranoside (35.18%)

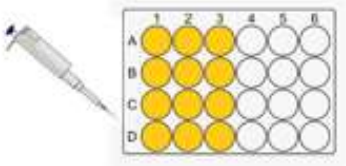


HPLC, Nexera system (Shimadzu), consisting of an autosampler SIL-30AC, a mass spectrometer QExactive Plus; Explice Plus C-18 column (150 mm × 3 mm, 3.5 µm film thickness), flow 0.4 mL/min. The column oven was thermostated at 40°C. The solvent is methanol with 2% acetic acid. MS scan range of m/z 100 to 1500 amu.

Results of dose finding cytotoxicity assay

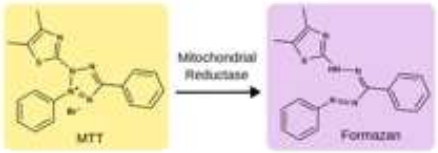
Method

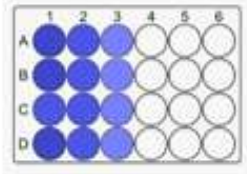
Viability assay



HaCaT cells, 10^6 cells in 96 well plate, 37 °C, 5% CO_2, 24 h, n=3

MTT as an indicator (570 nm)





MTD were determined to evaluate cytotoxicity (n=3)

Compound	Parameters	
	MTD*, weight %	Non-cytotoxic concentration range with a viability more than 80%, weight %
Aloe vera extract standardized for aloin	24.5	from 0.01 to 10.0
Trimethylglycine	39.0	from 0.01 to 10.0
Combination of Aloe vera extract and trimethylglycine (in a mass ratio 1:1)	18.0	from 0.01 to 10.0
Glyceryl glucoside	40.8	from 0.01 to 1.0

*MTD is a concentration providing viability at 70% in this test.

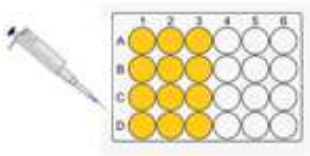
Aloe vera extract standardized for aloin, trimethylglycine and the combination of *Aloe vera* extract and trimethylglycine in a 1:1:1 mass ratio had a good cytotoxicity profile on epidermal cells.

Determining the MTD beforehand simplifies the interpretation of the efficacy assay by reducing the interferences generated by cytotoxicity of the chemicals.

Results of dose finding cytotoxicity assay

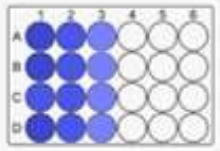
Method

Viability assay



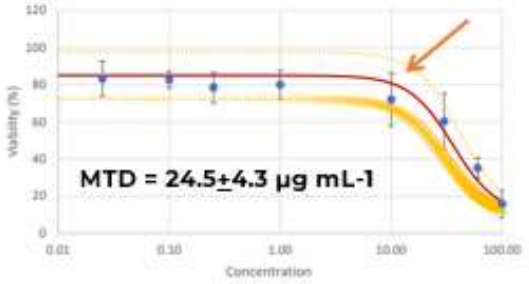
HaCaT cells, 10^6 cells in 96 well plate, 37 °C, 5% CO_2, 24 h, n=3

MTT as an indicator (570 nm)

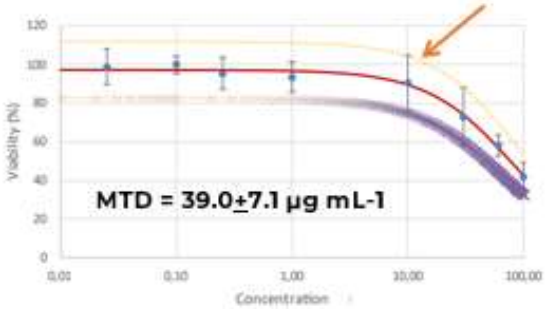


MTDs were determined to evaluate cytotoxicity (n=3)

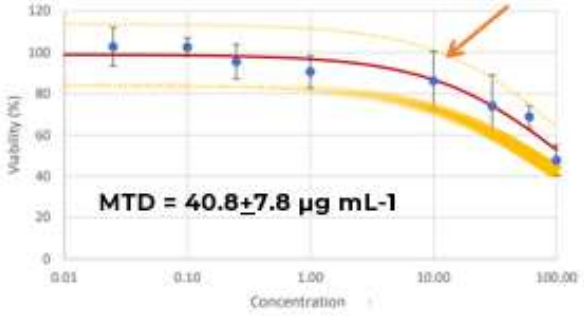
Aloe Barbadensis extract standardized for aloin



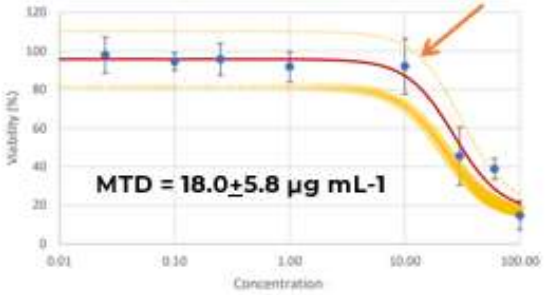
Trimethylglycine



Glyceryl glucoside



Combination of *Aloe Barbadensis* extract and trimethylglycine in a 1:1:1 mass ratio

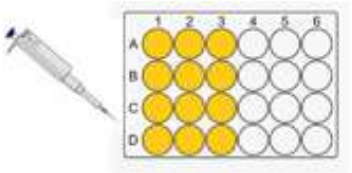


*MTD is a concentration providing viability at 70% in this test.
**Non-cytotoxic concentration range is related to viability more than 90% epidermal cells.

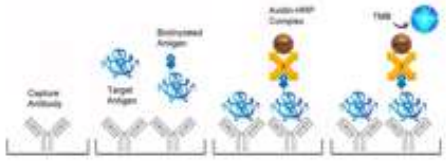
AQP3 determination in skin epidermis cells

Method

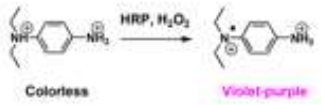
AQP3 quantification with Aquaporin 3 ELISA kit



HaCaT cells, 10^5 cells in 96 well plate, 37 °C, 5% CO2, 24 h, n=3



Horseradish peroxidase as an indicator (450 nm)



Average of AQP3 amount in skin epidermis (n=4)

Amount of AQP3, ng/mL				
Negative control (without any compound)	Compound in a concentration of 1 weight %			
	Aloe vera extract standardized for aloin	Trimethylglycine	Combination of *Aloe vera* extract and trimethylglycine (in a mass ratio 1:1)	Glyceryl glucoside (active control)
5.58±0.24	**6.71±0.29***	6.56±0.52	**12.20±0.37**	**6.89±0.36***
-	+20.25%	+17.56%	**+118.64%**	+23.48%

statistical significance in this test (*p<0.1; **p<0.05; ***p<0.01)

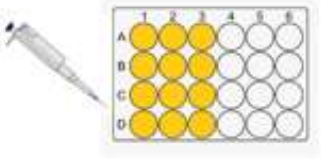
The combination of *Aloe vera* extract and trimethylglycine in a 1:1:1 mass ratio had a significant increase of AQP3 amount up to 119%, compared to the negative control.

It was evidence of synergetic activity between *Aloe vera* extract standardized and trimethylglycine in a 1:1:1 mass ratio.

AQP3 quantification in skin epidermis cells

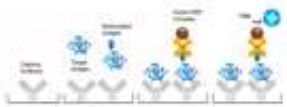
Method

AQP3 quantification with Aquaporin 3 ELISA kit

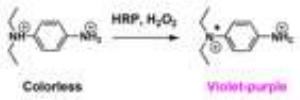


HaCaT cells, 10^6 cells in 96 well plate, 37 °C, 5% CO2, 24 h, n=3

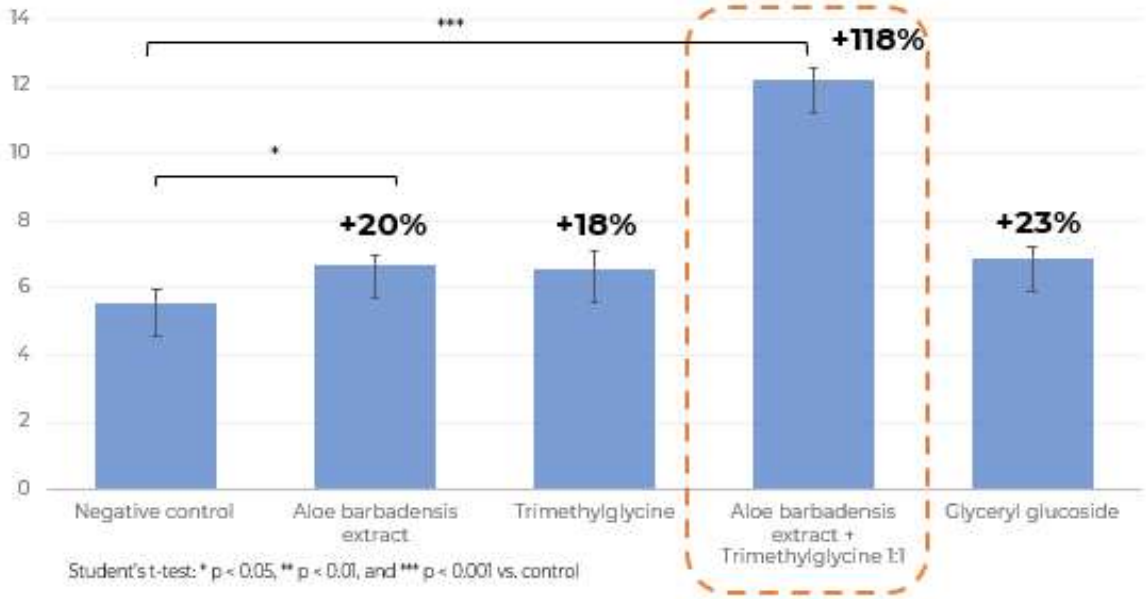
Addition substances in a concentration of 1 µg mL-1.



Horseradish peroxidase (450 nm)



Average of AQP3 amount (ng/mL) in skin epidermis (n=4)

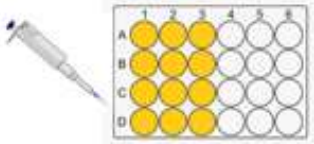


The combination of *Aloe barbadensis* extract and trimethylglycine in a 1:1 mass ratio had a significant increase of AQP3 amount up to 119%, compared to the negative control.

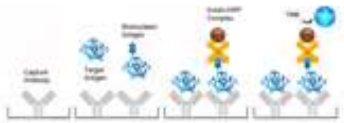
Determination the optimal ratio of phytochemicals in the novel combination

Method

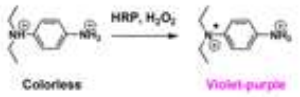
AQP3 quantification with Aquaporin 3 ELISA kit



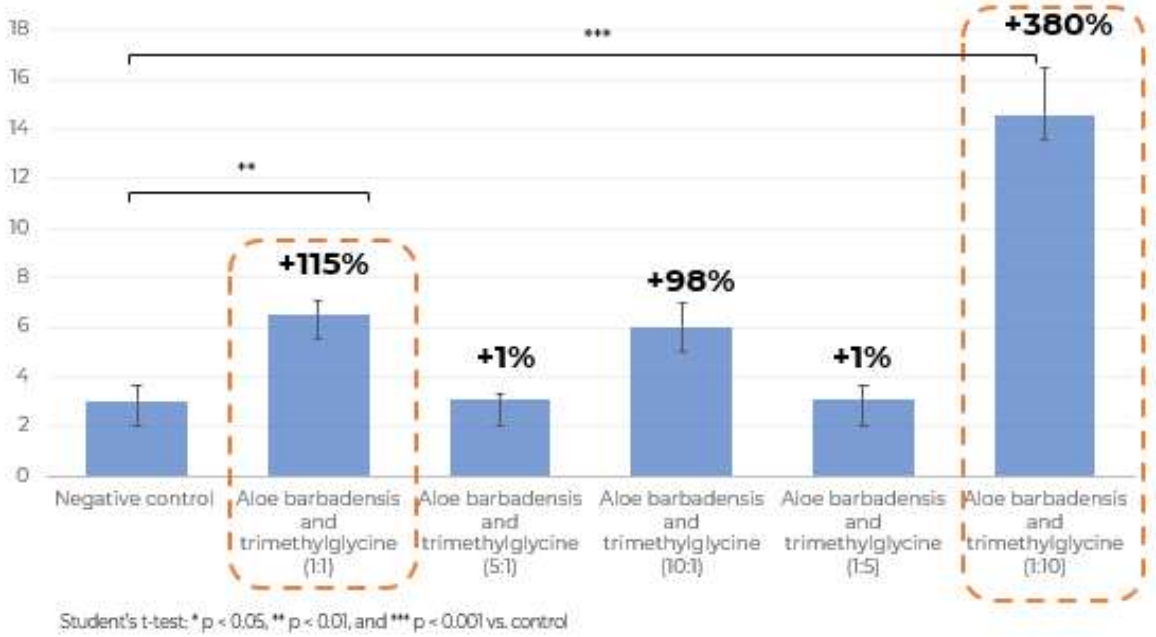
HaCaT cells, 10^6 cells in 96 well plate, 37 °C, 5% CO2, 24 h, n=3



Horseradish peroxidase (450 nm)



Average of AQP3 amount (ng/mL) in skin epidermis (n=4)



Student's t-test: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. control

The combination of *Aloe barbadensis* extract and trimethylglycine in a 1:10 mass ratio had the most significant increase of AQP3 amount up to 380%, compared to the negative control.

Results and future prospectives

1. The novel combination of plant origin was based on *Aloe barbadensis* extract standardized for aloin and trimethylglycine for increase of AQP3 amount in skin epidermis.
2. Aloin was a prevalent compound in *Aloe barbadensis* extract according HPLC-MS analysis.
3. The optimal mass ratio of *Aloe barbadensis* extract standardized for aloin and trimethylglycine in the novel combination was 1:10 according in vitro research.
4. This novel combination in a 1:10 mass ratio had the most significant increase of AQP3 amount in skin epidermal cells in comparison with negative control.

Try to find the reason why the ratios of compounds in the combination affect the AQP3 amount in epidermal cells

Research the AQP3 gene expression and AQP3 amount in the presence of the combination and single compounds ex vivo (skin explant model)

Investigate the possible influence of the combination on the proliferation of skin melanoma cells

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