

CEREUS GRANDIFLORUS FLOWER EXTRACT MODULATES GENES INVOLVED IN CIRCADIAN RHYTHM AND CELL MIGRATION TO FAVORIZE NIGHT CELL PROCESSES

Misset Gabrielle¹; Gueniche Audrey¹; Fagot Dominique²; Nègre Caroline³
¹L'Oreal Research and Innovation, Chevilly Larue, France ²L'Oreal Research and Innovation, Aulnay-sous-Bois, France ³YSL Beauty, Levallois, France

1 INTRODUCTION

Cereus grandiflorus belongs to the *Cactaceae* family (cactus). It is native from the desert regions of the Antilles, tropical America, and Mexico. Its flowers bloom with white petals only at night during six hours. This medicinal flower is traditionally used to treat heart diseases. It is rich in sugars such as glucose, fructose, amino acids, acids (citric, fumaric, malonic...), alkaloids (including cactine) and flavonoids which are known for their cosmetic properties. And phenolic compounds that may exert their effects through interactions with the biological clock system. [1]

It has been demonstrated that many metabolic pathways are under the control of clock genes [2], present in each cell of the body that function in a circadian and autonomous manner. It is well demonstrated that the release of melatonin during nighttime plays a role in the circadian rhythm and involves interaction with clock genes. [3]

At skin level, these clock genes control the rate and intensity of expression of genes involved in the quality of the skin barrier, in hydration [4], in defense and / or regeneration of the skin. Interestingly, during nighttime the circadian rhythm favorize the regeneration processes. [5]

The objective was to assess if *C. grandiflorus* extract might improve keratinocytes migration and modulate the circadian rhythm, as compared to melatonin.

2 MATERIALS & METHODS

EXTRACT OBTENTION

The extract is obtained from blossoms of *Cereus grandiflorus*. They are dried, then extracted with water and filtrated. Eventually the filtrate is concentrated by distillation. The developed extract of flower for cosmetic is a complex ingredient whom composition has been described, containing flavonols, organic acids, phenolic acids, sugars, nucleosides.

ARTIFICIAL HUMAN KERATINOCYTE WOUND HEALING MODEL

The keratinocytes were seeded in culture medium in a 96-well plate dedicated to migration analysis (ref. Platypus Oris™ Collagen I Coated Plate). The cells were treated with the *C. grandiflorus* extract during 24 hours of preincubation, the caches were removed then the cells were labeled with calcein-AM, incubated for 30 minutes and images were taken (T0). The medium was then replaced by test medium containing or not containing (control) the 100% *Cereus grandiflorus* extract at 2 doses (0,1mg/mL and 0,3mg/mL) to observe its action on cells migration at times 14, 18 and 24 hours. Positive reference of the test is EGF at 10ng/mL.

CIRCADIAN GENES ACTIVITY

The hydroglycerin extract concentrated at 1.4% of *C. grandiflorus* extract has been tested at 2 concentrations 1mg/mL and 3.3mg/mL on normal human keratinocytes, in comparison with melatonin at 66.7 µg/ml and 200 µg/ml, to observe the modulation of the *C. grandiflorus* extract on 14 circadian genes expression on basal situation during 48h via a qPCR.

Relative expression (% of control)	Classification of effects
> 200% and < 300%	Stimulation
> 150% and < 200%	Slight stimulation
> 50% and < 70%	Moderate inhibition
> 30% and < 50%	Inhibition

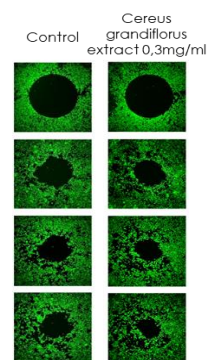
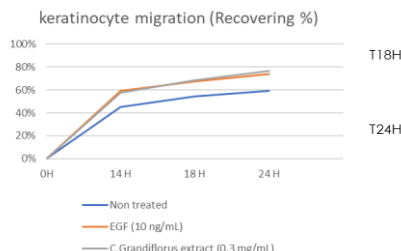
REFERENCES

- Avila-Román J, Soliz Rueda JR, Bravo F, Aragonès G, Suarez M, Arola-Arnal A, Mulero M, Salvadó M, Arola L, Torres-Fuentes C, Muguera B (2021). Phenolic compounds and biological rhythms: Who takes the lead?. *Trends in Food Science & Technology*.
- Dyar KA, Eckel-Mahan KL (2017) Circadian Metabolomics in Time and Space. *Front Neurosci*. 11:369.
- Pevet P, Challet E. (2011) Melatonin: both master clock output and internal time-giver in the circadian clocks network. *J Physiol Paris*. 105(4-6):170-82
- Matsunaga N, Itcho K, Hamamura K, Ikeda E, Ikeyama H, Furuichi Y, Watanabe M, Koyanagi S, Ohdo S (2014) 24-hour rhythm of aquaporin-3 function in the epidermis is regulated by molecular clocks. *J Invest Dermatol*. 134(6):1636-1644.
- Janich P, Touffighi K, Solanas G, Luis NM, Minkwitz S, Serrano L, Lehner B, Benitah SA (2013) Human epidermal stem cell function is regulated by circadian oscillations. *Cell Stem Cell*. 13(6):745-53.

3 RESULTS & DISCUSSION

ARTIFICIAL HUMAN KERATINOCYTE WOUND HEALING MODEL

In the artificial keratinocyte wound healing test, *C. grandiflorus* extract (0.3mg/mL) significantly stimulated cells migration with an average 128% over the 3 analysis times

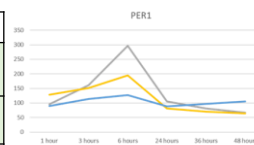


CIRCADIAN GENES ACTIVITY

The qPCR array on circadian genes demonstrated that both, melatonin and *C. grandiflorus* extract modulate 6 circadian genes, including PER1 and the melatonin receptors, following similar patterns:

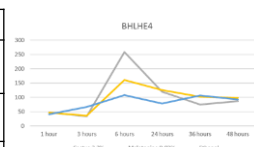
PER 1

In % vs control	1H	3H	6H	24H	36H	48H
Melatonin at 200 µg/ml in ethanol	129	152	195	81	71	64
C. Grandiflorus extract at 3.3mg/mL	96	161	296	105	81	67
Ethanol	90	114	127	89	97	105



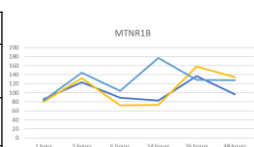
BHLHE40

In % vs control	1H	3H	6H	24H	36H	48H
Melatonin at 200 µg/ml in ethanol	46	37	161	126	102	98
C. Grandiflorus extract at 3.3mg/mL	48	34	258	120	75	86
Ethanol	41	66	108	79	106	93



MTNR1B

In % vs control	1H	3H	6H	24H	36H	48H
Melatonin at 200 µg/ml in ethanol	80	132	72	73	158	134
C. Grandiflorus extract at 1mg/mL	86	123	89	83	137	97
Ethanol	83	145	104	177	128	128



4 CONCLUSION

The efficacy of *C. grandiflorus* flower extract on skin is poorly documented, those new data on keratinocytes allow us to complete previous generated data and go further in the understanding of this new active. Interestingly this active showed a capacity to promote epidermal regeneration processes by increasing migration of keratinocyte on the wound model. Moreover, the results of *C. grandiflorus* flower extract on circadian gene expression in parallel of Melatonin show interesting action to promotes potential skin night processes, by modulating gene expression with similar pattern as melatonin.