Skin mechanoreceptors for gentle touch and proprioception are connected to oxytocin peripheral system.

Labarrade, Florian[1]; Perrin, Armelle[1]; Le Mestr, Audrey[1]; Plaza, Christelle[1]; Meyrignac, Celine[1]; Capallere, Christophe[1]; Botto, Jean-Marie[1]; **Imbert, Isabelle[1]**

[1]Ashland, Global Skin Research Center, Sophia-Antipolis, France

* Imbert Isabelle, 655, route du Pin Montard 06904 Sophia Antipolis, iimbert@ashland.com

Abstract

Background: The human skin is a sensory organ, sensations from our environment are gathered by nerve fibers. Nerve fibers are found in the dermis and, as free nerve endings in the epidermis. Touch and proprioception involve piezo1 and piezo2 mechanoreceptors. Keratinocyte express piezo1, and mediate touch sensation by detecting and encoding tactile information to sensory neurons. Moreover, pleasant touch has been shown to trigger oxytocin release, a molecule linked to increased levels of social interaction, well-being, and anti-stress effects. In this study, we investigated the role of piezo1 in skin barrier function, and the relationship with the oxytocinergic pathway.

Methods: We have screened various plant extracts for their ability to modulate piezo1 mechanotransducer, and the oxytocinergic pathway. Our results showed that piezo1 expression in the epidermis decreased with age. Expression of piezo1, E-cadherin, oxytocin and an its receptor OXTR were monitored by immunohistochemistry and ELISA assay. Antagonist Dooku1 was used for piezo1 receptor inhibition.

Results: Our results showed that piezo1 expression in the epidermis decreased with age. Moreover, inhibition of piezo1 in *ex vivo* skin, by a selective antagonist, significantly compromised the integrity of cellular junctions, and the skin peripheric oxytocinergic pathway. The application of a botanical extract was observed to preserve the expression of piezo1, oxytocin and its receptor OXTR. Finally, application of the botanical extract on keratinocytes, was associated with an increase of innate immune function.

Conclusion: These results highlighted the importance of maintaining piezo receptors in skin, to preserve the mechanical communication between cells and their environment. In this work, we provide knowledge on existing bond between mechanosensation and the neuroendocrine–immune network.

Keywords: (Oxytocin; Mechanoperception; PIEZO1; Sensory).

Introduction.

Age-related changes in skin mechanics have a major impact on the aesthetic perception of skin. Wrinkles, loss of firmness and elastic rebound degrades the perception of skin appearance and feel. In skin, multiple mechanisms exist to sense, transduce, and transmit force. These mechanisms include mechanosensitive ion channels (e.g., Piezo channels), and E-cadherin-based cell-cell adhesions. Mechanical forces drive the modelling of tissues, this relies on the transmission of forces between cells by *adherens* junctions. *Adherens* junctions mediate strong cell-cell adhesion and mediate transduction of mechanical signals between cells, which govern collective dynamics. Mechanistically, the adhesive extracellular domain of E-cadherin interacts with the cap domain of Piezo1 [1]. E-cadherin potentiates the mechanosensitivity of the Piezo1 channel [1]. E-cadherin integrates mechanotransduction as they are under constitutive actomyosin-generated tension that is increased at cell–cell contacts [2]. Activation of the piezo1 receptor converts physical force into biochemical information and is involved in maintaining of tissue organization. Thanks to piezo1, epidermal keratinocytes mediate touch sensation by detecting and encoding tactile information to sensory neurons [3]. Keratinocyte expression of piezo1 is critical for normal sensory afferent firing and behavioral responses to mechanical stimuli [4]. Pleasant touch plays a crucial role in behavior and social communication. Low intensity, non-noxious, stimulation of cutaneous somatosensory nerves has been shown to trigger oxytocin release and is associated with increased social motivation. Oxytocin is a key modulator in regulating social behavior and affective processing [5]. Oxytocin is released in response to affective touch and may act to facilitate the rewarding effects of social touch. More specifically, it has been reported that stroking touch and massage facilitate endogenous oxytocin release. Our research suggests that the piezo1/E-cadherins interactions should be linked to activation of the oxytocinergic system, including its powerful anti-aging effect [6], anti-stress effect [7], and immune defense strengthening [8].

Materials and Methods.

The extraction process of the *Jasminum grandiflorum* flower uses the phytobiome of jasmine flowers to orchestrate phytocompounds biotransformation, without any addition of exogenous microorganisms' source. Expression of piezo1, E-cadherin, and oxytocin were monitored by immunohistochemistry, qPCR, and ELISA assay. Antagonist Dooku1 was used for piezo1 receptor inhibition.

Results.

Piezo1 expression was characterized in human keratinocytes, in *ex vivo* skin, and in reconstructed human epidermis (RHE) (Figure 1). Piezo1 expression was detected in the cytoplasm and in all layers of the epidermis. Few dermis cells also expressed the mechano-receptor. A RHE model confirmed piezo1 expression pattern in the epidermis (Figure 1).

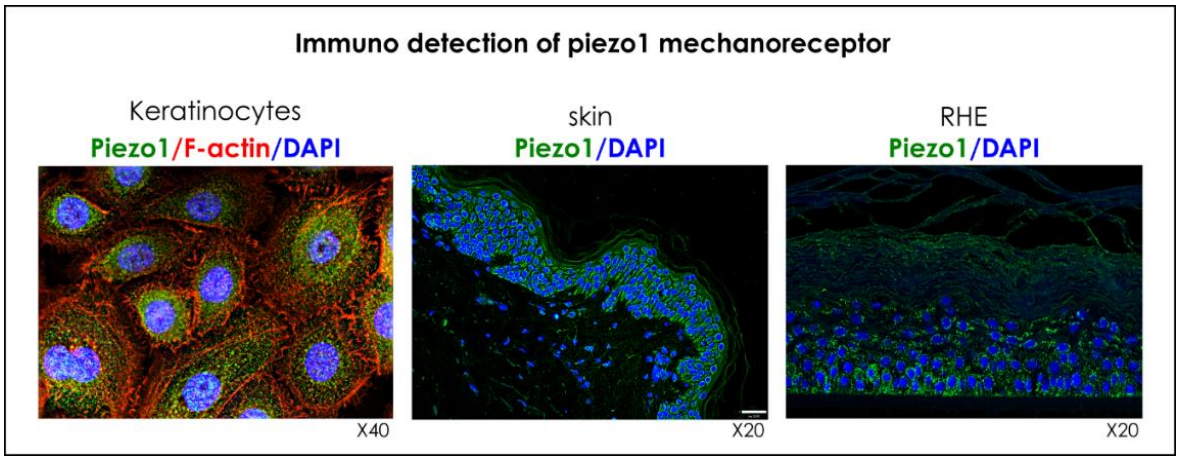

Figure 1: Immuno detection of piezo1 (green), F-actin cytoskeleton (red), and nucleus (blue). (A) In cultured primary keratinocytes. (B) In *ex vivo* human skin biopsies. (C) In reconstructed human epidermis (RHE).

Expression studies using RHE models have revealed that the expression of piezo1 decrease can be associated with aging (Figure 2A). OXTR expression was then investigated in an induced-senescent RHE model (Foxo3 silenced, Foxo3 is a gene consistently annotated as a human longevity gene). We observed a decreased expression of OXTR (-29%) in the senescent RHE (Figure 2B). Both pathways are linked to expression changes in relation to aging.

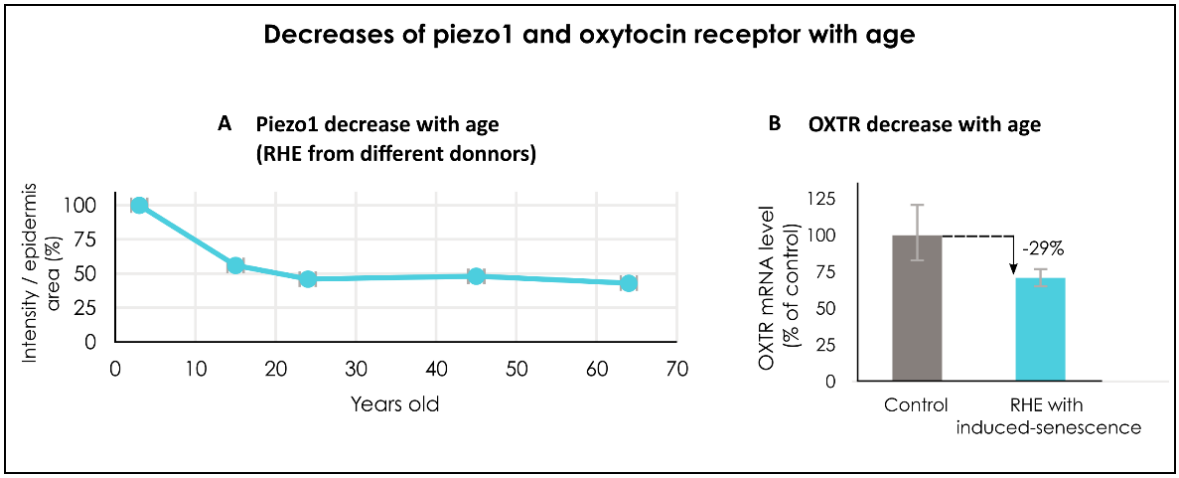

Figure 2: Expression of piezo1 protein and OXTR mRNA with aging. (A) Quantification of Piezo1 expression in reconstructed epidermis. Keratinocytes from donors with various ages were used. (B) Quantification of OXTR mRNA by qPCR in RHE. Foxo3 expression was silenced with a siRNA to induce the senescence.

A piezo1 antagonist called Dooku1 was applied on skin biopsies for 48 hours with 2% of *J. grandiflorum* extract. Hematoxylin and eosin stains showed that piezo1 inhibition caused

epidermal sagging, accompanied by structural damages. Biopsies treated with *Jasminum grandiflorum* extract showed a preserved tissue morphology, without damages, maintaining its mechanics intact.

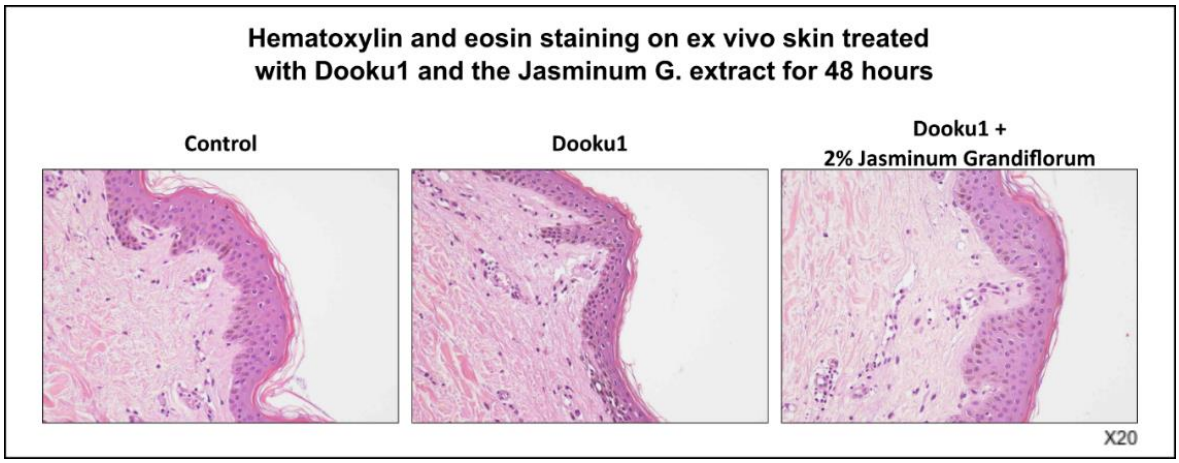

Figure 3: Hematoxylin and eosin staining of *ex vivo* skin treated with Dooku1 and the *Jasminum grandiflorum* extract at 2% for 48 hours.

Inhibition of piezo1 activity by the antagonist Dooku1 decreased the expression level of E-cadherins (-31% compared to the control). Analysis of biopsies treated with *Jasminum grandiflorum* extract showed preservation of E-cadherin expression. The antagonist Dooku1 caused a decrease in oxytocin (-34% compared to the control). Application of the *Jasminum grandiflorum* resulted in preservation of oxytocin level compared to Dooku1 condition.

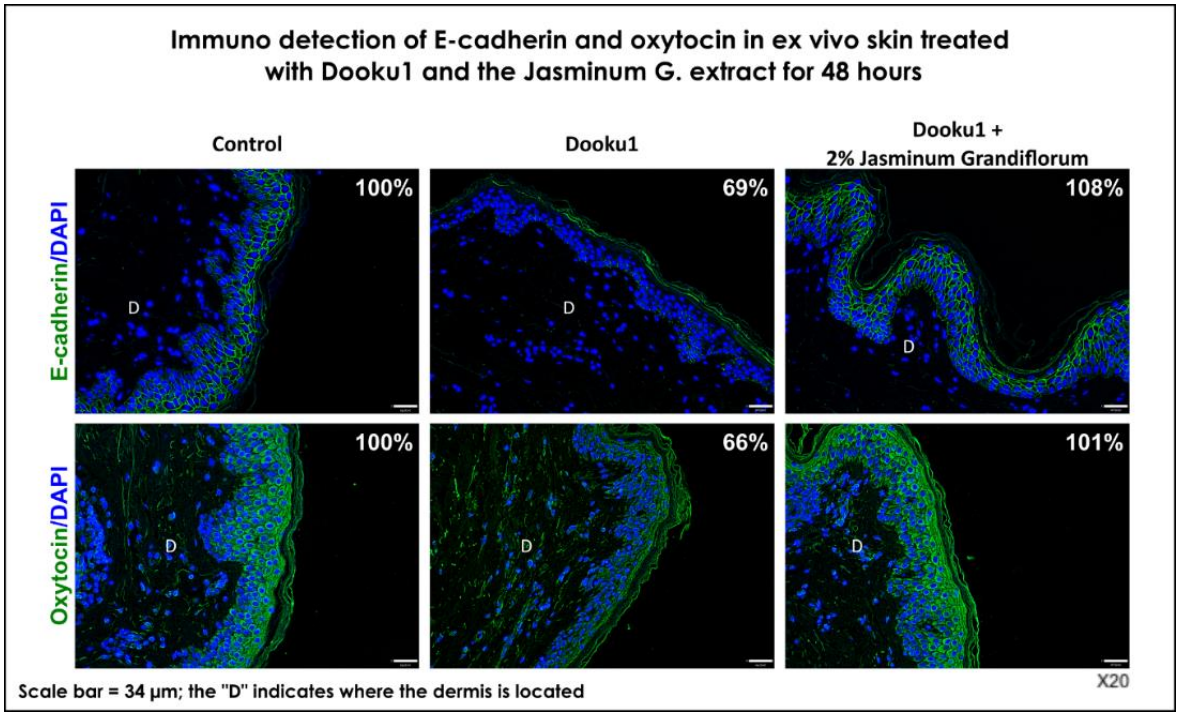

Figure 4: Immunodetection of E-cadherin and oxytocin in *ex vivo* human skin treated with Dooku1, and the *Jasminum grandiflorum* extract at 2% for 48 hours.

Oxytocin is a secreted molecule and an inhibition of piezo1 activity reduced its release in the *ex vivo* skin culture media. Application of the *Jasminum grandiflorum* extract resulted in preservation of oxytocin level compared to Dooku1 condition (Figure 5).

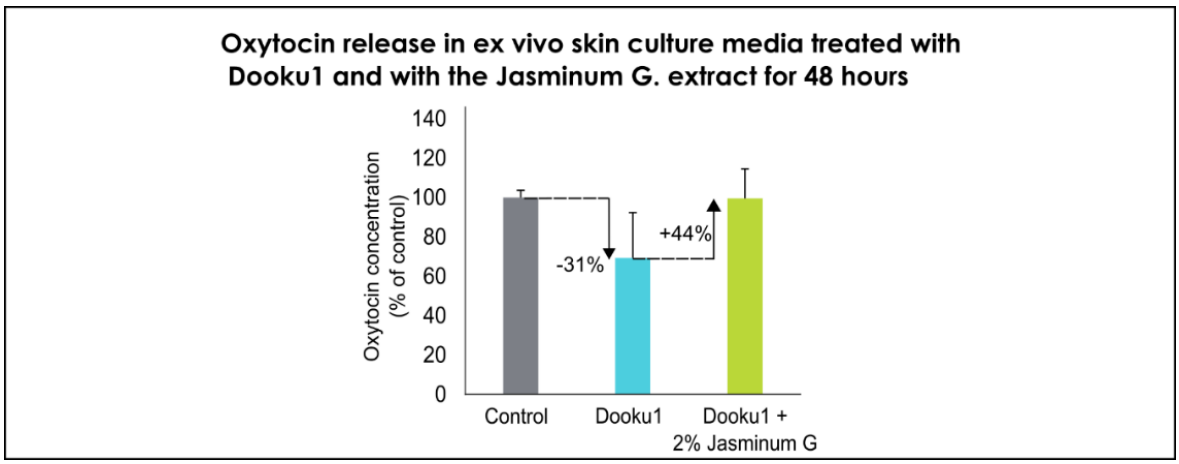

Figure 5: ELISA quantification of oxytocin released in the *ex vivo* skin culture media. ELISA was performed in the collected culture media of *ex vivo* skin biopsies treated with Dooku1 and the *Jasminum grandiflorum* extract at 2% for 48 hours.

Application of the *Jasminum grandiflorum* extract increased OXTR expression. 48 hours after treatment, we observed an increase of OXTR in *ex vivo* skin (Figure 6A), and an increase of OXTR mRNA in cultured keratinocytes (Figure 6B).

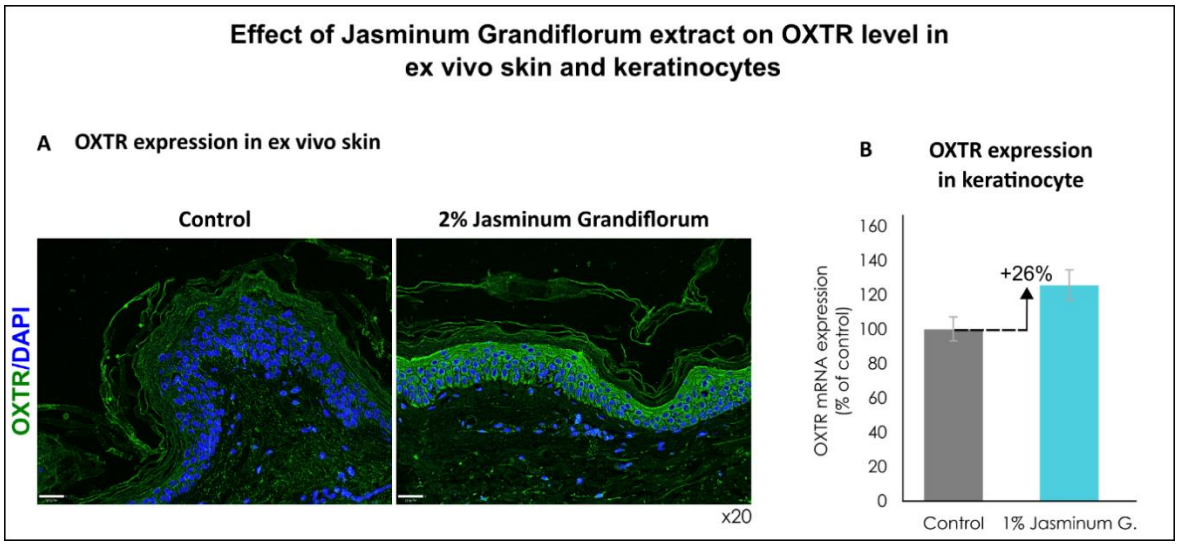

Figure 6: OXTR expression after treatment with the *Jasminum grandiflorum* extract. A) Expression of OXTR in *ex vivo* skin biopsies treated with 2% *Jasminum grandiflorum* extract for 48 hours. B) Quantification of OXTR mRNA by qPCR in keratinocytes treated with 1% *Jasminum grandiflorum* extract for 48 hours.

Oxytocin exerts stress-buffering effects to inhibit the stress-induced HPA activity and decreases cortisol levels. 48 hours after treatment with the *Jasminum grandiflorum* extract, we observed a reduced 11β-HSD1 mRNA expression in keratinocytes. 11β-Hydroxysteroid dehydrogenase type 1, also known as cortisone reductase convert cortisone into the cortisol (Figure 7).

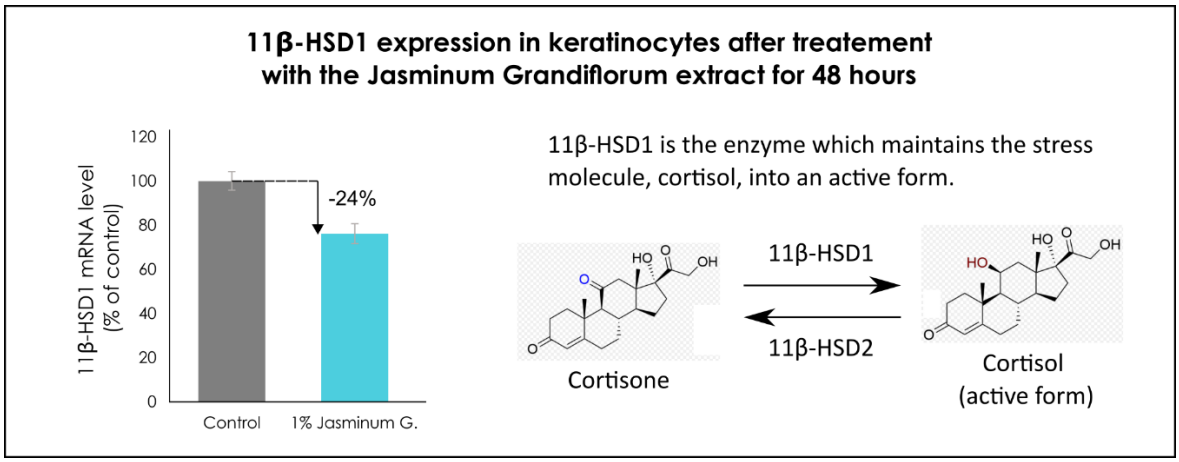

Figure 7: Quantification of 11β-HSD1 mRNA by qPCR in keratinocytes treated with 1% *Jasminum grandiflorum* extract for 48 hours.

Oxytocin has immune-regulating functions. We observed in cultured keratinocytes treated with the *Jasminum grandiflorum* extract for 48 hours, an increase in viperin level (Figure 8). Viperin is an interferon-inducible protein that inhibits the replication of a variety of viruses.

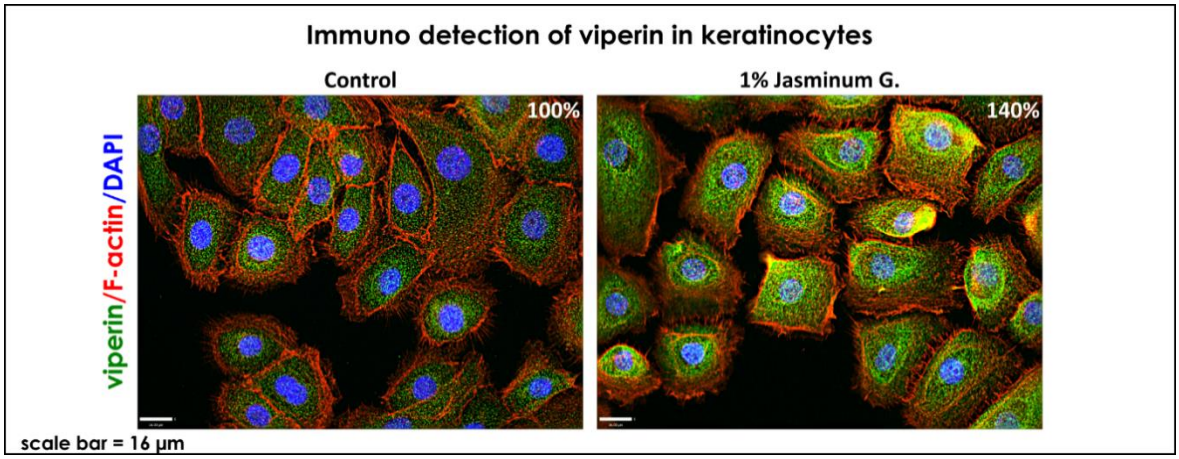

Figure 8: Immuno detection of viperin (green) F-actin cytoskeleton (red), and nucleus (blue) in cultured keratinocytes treated with 1% *Jasminum grandiflorum* extract for 48 hours.

Conclusion.

In conclusion, we provide evidence for the presence in the human skin of piezo1 receptor by immunohistochemistry. We observed an up-regulation of E-cadherin, oxytocin, and its receptor OXTR in *ex vivo* skin, after *Jasminum grandiflorum* extract application. The *Jasminum grandiflorum* extract has been shown to preserve the skin mechanics in presence of Dooku1 (piezo1 antagonist). For the first time, our results have shown that the use of a phytofermented cosmetic ingredient derived from *Jasmine grandiflorum* would biologically activate sensors, release molecules such as oxytocin into the skin, providing antistress and immune reinforcement.

Conflict of Interest Statement. NONE.

References.

1. Wang J, Jiang J, Yang X, Zhou G, Wang L, Xiao B. Tethering Piezo channels to the actin cytoskeleton for mechanogating via the cadherin-β-catenin mechanotransduction complex. Cell Rep. 2022;38(6):110342. doi:10.1016/j.celrep.2022.110342

2. Borghi N, Sorokina M, Shcherbakova OG, et al. E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. Proc Natl Acad Sci U S A. 2012;109(31):12568-12573. doi:10.1073/pnas.1204390109

3. Dance A. The quest to decipher how the body's cells sense touch. Nature. 2020;577(7789):158-160. doi:10.1038/d41586-019-03955-w

4. Moehring F, Mikesell AR, Sadler KE, Menzel AD, Stucky CL. Piezo1 Mediates Keratinocyte Mechanotransduction. bioRxiv; 2020. PPR: PPR189392

5. Oettl LL, Ravi N, Schneider M, et al. Oxytocin Enhances Social Recognition by Modulating Cortical Control of Early Olfactory Processing. Neuron. 2016;90(3):609-621. doi:10.1016/j.neuron.2016.03.033

6. Benameur T, Panaro MA, Porro C. The antiaging role of oxytocin. Neural Regen Res. 2021;16(12):2413-2414. doi:10.4103/1673-5374.313030

7. Grahn P, Ottosson J, Uvnäs-Moberg K. The Oxytocinergic System as a Mediator of Anti-stress and Instorative Effects Induced by Nature: The Calm and Connection Theory. Front Psychol. 2021;12:617814. Published 2021 Jul 5. doi:10.3389/fpsyg.2021.617814

8 Li T, Wang P, Wang SC, Wang YF. Approaches Mediating Oxytocin Regulation of the Immune System. Front Immunol. 2017;7:693. Published 2017 Jan 10. doi:10.3389/fimmu.2016.00693