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*Original Research*

# Protective Properties of Botanical Extracts against 5G Radiation-induced Damage to Human Skin, as Demonstrated in Preliminary Data from a Keratinocyte Cell Culture Model

Fabien Havas[1], Moshe Cohen[1], Shlomo Krispin[2], Joan Attia-Vigneau[3,*]

[1]R&D, IFF Ltd. – Lucas Meyer Cosmetics, 8122503 Yavne, Israel
[2]Marketing, IFF Ltd. – Lucas Meyer Cosmetics, 8122503 Yavne, Israel
[3]R&D, IFF Ltd. – Lucas Meyer Cosmetics, 31036 Toulouse, France
*Correspondence: joan.attia@lucasmeyercosmetics.com (Joan Attia-Vigneau)
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**Abstract**

**Background**: Next-generation 5G communication technology involves increasing use of 3–100 GHz wireless bands in population centers. Though still non-ionizing, this implies higher radiation energy vs. existing bands. The range is also shorter, needing more numerous emitters, closer to the user—resulting in higher electromagnetic energy exposure. With no universal consensus regarding exposure risks, there is some concern among the public and the scientific community, following indications that 5G radiation can impact immune function, trigger inflammatory responses, and influence expression of genes affecting protein folding, oxidative stress, tissue/extracellular matrix (ECM) matrix turnover, and more. This work aims at identifying botanical extracts for protection of human skin from these impacts, based on a preliminary cell culture-based model. **Methods**: We irradiated human epidermal keratinocytes at 6 GHz, evaluating effects on Interleukin1-$\alpha$ (IL1-$\alpha$), a key inflammatory cytokine; TIMP metallopeptidase inhibitor 1 (TIMP1), shown to inhibit collagenase; Angiopoietin-like protein 4 (ANGPLT4), which plays a role in wound healing and epidermal differentiation; and S100 calcium-binding protein A9 (S100A9), involved in immune recruitment during injury, by enzyme-linked immunosorbent assay (ELISA) and immunostaining. We next used this model to identify substances able to mitigate the effects of 5G irradiation, through the evaluation of the influence of treatment by one of several botanical extracts on the observed effects of 5G irradiation. **Results**: After a remarkably short 1-h exposure, clear effects on keratinocyte function were observed: increased inflammatory cytokine IL1-$\alpha$; reduced collagenase inhibitor TIMP1; increased wound healing/differentiation facilitator ANGPLT4; and increased SA100A9, involved in immune recruitment during injury. On this basis, we then showed the protective effects of selected botanical extracts, capable of reducing the increase in IL1-$\alpha$ induced by 5G exposure, possibly in part due to anti-inflammatory and anti-oxidant properties of compounds present in these extracts. **Conclusions**: Our results show a clear influence of 5G irradiation on the keratinocytes, possibly indicating injury and damage responses. What's more, we showed how these preliminary data can be used to identify botanical extracts capable of offering some protection against these effects for users of 5G technology, e.g., when employed as active ingredients in protective cosmetic applications.

**Keywords:** 5G; radiation; skin; inflammation; aging; *in vitro*; botanical; cosmetic; extract

## 1. Introduction

5G is shorthand for the incoming fifth generation technology standard for cellular wireless communication networks, whose deployment started worldwide ca. 2019. 5G uses electromagnetic, non-ionizing radiation in several frequency ranges from under 6 GHz and up to 100 GHz [1]. The use of higher frequency bands allows for wider data transfer volumes and higher transfer speeds (more "broadband" communication). 5G technology has thus been built on the use of millimeter waves, significantly shorter than what was used by previous cellular communication generations. As one of the results of this change, 5G has a shorter effective range, and therefore the technology requires a higher number of smaller cells, with a higher number of emitter stations [2]. These emitters would be placed closer together compared to the architecture of previous generations, and necessarily also closer to the end user (up to tens or hundreds of meters [3]). The creation of a ubiquitous network composed of numerous 5G emitters in close proximity to the general population will naturally lead to an increase in exposure to artificial electromagnetic radiation. While the radiation involved remains non-ionizing, there still exists some concern in the general public that the exposure to higher energies may lead to adverse health effects.

While a detailed discussion of the regulatory field is out of scope here, it should be noted that exposure limits are unclear, and sources (including regulatory/advisory bodies) have yet to reach any overall alignment and range from 1 to 20 mW/cm$^2$ [4,5]. For instance, some recommended general public and occupational exposure limits are available from the WHO, based on recommendations from bodies such as the International Commission on Non-Ionizing Radiation Protection (ICNIRP) or The Institute of Electrical and Electronics Engineers [4,6]. This is further complicated

by the extreme variability of real-life exposure, resulting from a vast range of device types and densities, geometries, distances, to name just a few parameters which will vary from environment to environment (e.g., urban to suburban) and between individual cases of nominally similar environments (e.g., downtown New York City vs. downtown Boise, Idaho).

Further, at the biological level a definite consensus is also still difficult to identify among the scientific community regarding possible hazards of exposure to this range of Electromagnetic radiation (EM radiation) [7,8]. As a starting point, the very fact that millimeter waves have been used for therapeutic purposes indicates at least the potential for interactions between these waves and biological tissue [9]. Several studies seem to suggest that there may in fact be potential adverse effects [10–13]. Investigating the effect of millimeter waves on rat dermis, Millenbaugh *et al*. [11] showed aggregation of neutrophils in vessels, degeneration of stromal cells, and breakdown of collagen, and observed changes in up to 58 genes at 24 h, including genes associated with regulation of transcription, protein folding, oxidative stress, immune response including chemokine activity, and tissue matrix homeostasis including extracellular matrix structure. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) confirmed up-regulation of *Hspa1a, Timp1, S100a9, CCL2*, and *Angptl4*. According to these authors, these results indicate that prolonged exposure to high-frequency millimeter waves "causes thermally related stress and injury in skin while triggering repair processes involving inflammation and tissue matrix recovery". Based on a study on HaCat keratinocytes, Le Pogam *et al*. [13] suggested that millimeter waves affect biomembrane permeability, and showed drastic changes in the extracellular metabolomic profile. Szabo *et al*. [12] suggested that exposure of human skin to millimeter waves can trigger the activation of basal keratinocytes (a key component of the epidermis), resulting in elevated Interleukin1-$\beta$ (IL1-$\beta$) levels, hinting at inflammatory processes. Soubere Mahamoud *et al*. [14] may have shown a long-term exposure effect, in a study where while millimeter waves alone seemed to have no acute effect, co-exposure to millimeter waves and 2-deoxyglucose (2dG, a glycolysis inhibitor) had additional effects relatable to a bioenergetic stress response, including changes in the expression of Suppressor of cytokine signaling 3 (*SOCS3*), Sprouty RTK Signaling Antagonist 2 (*SPRY2*), Tribbles Pseudokinase 1 (*TRIB1*), non-canonical poly(A) polymerase *FAM46A*, Cysteine And Serine Rich Nuclear Protein 1 (*CSRNP1*), and Protein Phosphatase 1 Regulatory Subunit 15A (*PPP1R15A*), encoding transcription factors or cytokine pathway inhibitors [14]. It was suggested that these results raise the question of a potential impact of long-term or chronic millimeter wave exposure on metabolically stressed cells. Remarkably, Betzalel *et al.* [15] have shown that sweat ducts may act like helical antennas receptive to millimeter waves—raising the possibility that the energy thus captured in an unexpectedly focused manner may have more negative downstream effects on the surrounding tissue. It is important to note, in contrast, that not all studies have shown negative effects – indeed, some studies show no effect on biological systems [16]. While some reviews [17,18] suggest the possibility of systemic effects reaching well beyond peripheral tissues such as the skin, including that of correlation between radiofrequency EM exposure and several types of cancer; and while some researchers are calling for the application of the precautionary principle in relation to the widening use of this technology [10], is it difficult to identify any reliable real-life occupational studies. Indeed, it may be said that the main consensus on this issue is that more research is needed [16,17,19].

In recent years, considerable dermatological and cosmetic research has been devoted to the (negative) effects of skin exposure to various parts of the electromagnetic spectrum, from Ultraviolet (UV) and high-energy visible (blue) light, and down to infrared [20–23]. In light of the above, we believe there is a need to expand this focus to include the investigation of the effects of 5G radiation on skin, with a view to helping anticipate the needs of an increasingly exposed population. More specifically, in light discussion above, we believe that the identification of substances capable of protecting human skin from any negative effects of 5G radiation would be a valuable contribution to the field—this was therefore the main ultimate goal this work.

We thus report here on a preliminary *in-vitro* cell-based model, exposing a keratinocyte culture to 5G radiation (at 6 GHz) over relatively short time scales, and on the effects of this exposure on the cell culture. The endpoints examined, selected based on the precedents cited above and so as to represent a relevant set of tissue damage indicators, included: IL1-$\alpha$, a key inflammatory cytokine; TIMP metallopeptidase inhibitor 1 (TIMP1), shown to inhibit collagenase; Angiopoietin-like protein 4 (ANGPLT4), which plays a role in wound healing and epidermal differentiation; and S100 calcium-binding protein A9 (S100A9), involved in immune recruitment during injury [24–29]. While this model will not answer on its own the myriad open questions around the effects of millimeter wave irradiation on biological systems, we believe that it will have relevance in shedding some additional light on the processes in skin cells exposed to this radiation; and, that it will enable us to reach the practical goal of identifying applicable means of preventing or mediating these effects in real life situations for users of 5G and others so exposed, using readily available equipment and materials.

In pursuit of that specific goal, we also report on the use of the preliminary model described here for the identification of substances able to mitigate the effects of 5G irradiation, e.g., for use as active materials in topical formulations. Marine and terrestrial plant and bacterial extracts have been used to protect the skin from a wide range

of stressors, including various parts of the electromagnetic spectrum [30–35]. This protection effect has often been attributed to anti-oxidative and anti-inflammatory properties of these plant extracts [36,37]. Following these precedents, we report here on the protective effects observed with three botanical extracts using this same model: an aqueous extract of the aerial parts of *Cistus incanus*, a Mediterranean shrub rich in polyphenolic metabolites (notably flavanols, including the myricetin glycoside myricitrin), and with demonstrated anti-inflammatory and antioxidant properties, *inter alia*; an extract of the flower of *Trifolium pratense* (clover), a widespread medicinal herb also known to possess anti-inflammatory and anti-oxidant properties, containing the flavonoid biochanin A; and an extract of the extremophile *Alteromonas sp.*, whose major component is a soluble exopolysaccharide and which was shown to enhance keratinocyte renewal *in vitro* [38–47].

## 2. Materials and Methods

### *2.1 Effects of 5G Radiation on an Epidermal Keratinocyte Culture*

Primary Normal Human Epidermal Keratinocytes (NHEK) (Promocell C-12001, Heidelberg, Germany; these cells are tested by the supplier for cell morphology, adherence rate, and cell viability. Immunohistochemical tests for specific cytokeratins are carried out for each lot. Growth performance is tested through multiple passages up to 15 population doublings (PD) under culture conditions without antibiotics or antimycotics. Finally, cells are tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1, HTLV-2, fungi, bacteria, and mycoplasma.) were cultured at 37 °C in keratinocyte basal medium (Promocell C-20216, Heidelberg, Germany) supplemented with 0.06 mM $Ca^{2+}$ (Promocell C-34005, Heidelberg, Germany) and a keratinocyte-specific supplement mix (Promocell C-39016, Heidelberg, Germany), under a humidified atmosphere containing 5% $CO_2$, so as to reach 40–60% confluency at the time of irradiation. The culture medium was renewed just before irradiation, and the cells were exposed to 5G bandwidth radiation using a N5172B EXG X-Series RF Vector Signal Generator (Keysight Technologies, Santa Rosa, CA, USA), placed 9.5 cm above the cell culture, at 6 GHz, 30 dBm, 20 mW/$cm^2$ (based on the recommended exposure limits for currently widespread 5G equipment types in guidelines quoted above: the guidelines on electromagnetic field exposure by ICNIRP [4] specify a maximum power density of 10 mw/$cm^2$ for occupational exposure, averaged over an area of 4 $cm^2$; when averaging for smaller areas on the order of 1 $cm^2$, such as for small diameter/focal beam exposure as is the case for the irradiated area in this study, the maximum power density is 20 mw/$cm^2$), for 1 h, 2 h, 4 h, 6 h or 24 h. A control leg was left untreated except for parallel renewal of the culture medium. The cells were then fixed with methanol for 5 minutes at –20 °C, and frozen.

The cells were blocked with PBS (Sigma Aldrich P3813-10PAK, St Louis, MO, USA) containing 0.001% Tween-20 (Sigma Aldrich P1379, St Louis, MO, USA), 1% BSA (Sigma Aldrich A9085, St Louis, MO, USA) and 10% goat serum (Sigma Aldrich, G9023, St. Louis, MO, USA) during 1 h and then incubated overnight at 4 °C with: anti-TIMP1 mouse monoclonal antibody (Invitrogen MA1-773, Waltham, MA, USA, diluted 1/2000 in blocking solution); anti-ANGPTL4 rabbit monoclonal antibody (Invitrogen 701155, Waltham, MA, USA; diluted to 1/500 in blocking solution); or anti-S100A9 rabbit polyclonal antibody (Invitrogen PA5-79949, Waltham, MA, USA; diluted to 1/500 in blocking solution). Secondary antibodies (AlexaFluor® AF488 and AF594, Thermo Fisher, Waltham, MA, USA) were used for immunofluorescence staining, which was assessed by microscopical observation. Manual scaling was carried out per field, taking into account all cells in the field. IL1-$\alpha$ was quantified by enzyme-linked immunosorbent assay (ELISA) (human IL1-$\alpha$ kit, Cayman Chemical 583301, Ann Arbor, MI, USA). Four independent biological replicates (4 sampled wells) were included for each set of conditions.

Data analysis was performed using GraphPad Prism 9 (GraphPad Software, Inc., Boston, MA, USA). Statistical tests were selected on the basis of normality and lognormality tests: a parametric test was applied when the normality was positive, and a non-parametric test was used when the normality was negative. For this study, an unpaired Student *t*-test was applied. Data in the figures is shown as mean +/- Standard Deviation, with *p*-values appearing on brackets.

### *2.2 Assessment of Protective Effect of Botanical Extracts*

#### 2.2.1 Materials

Several botanical extracts were used in demonstrating possible protective effects against the effects of 5G radiation, including:

Aqueous extract of *Cistus incanus* aerial parts (IBR-Chill™, IFF—Lucas Meyer Cosmetics, Yavne, Israel): Aerial parts of Cistus incanus were harvested by hand in Southern Israel (by IFF—Lucas Meyer Cosmetics staff members) [38]. The dried and ground biomass was then extracted in heated water. Solids were separated, and the resulting liquid was filtered, yielding a crude extract which was diluted 1:1 in glycerin (Interaxion C50380681, Deyme, France) and preserved (with 1% Sharomix HP, Sharon Laboratories, Ashdod, Israel). The final extract's color is light brown to brown, with a pH of 4.5–6.5. The extract's content of the flavonoid glycoside myricitrin was titrated by high-performance liquid chromatography (HPLC) (Agilent, Santa Clara, CA, USA: 1100 series with G1315A diode array detector and Poroshell Phenyl Hexyl 150 × 4.6 mm, 2.7 µm column). The extract was diluted ×10 in HPLC grade water (deionized and irradiated with Direct-Q 5 UV, Merck-Millipore, Burlington, MA, USA), and filtered (0.22 µm) before injection. A myricitrin standard

(Sigma-Aldrich 67268, St Louis, MO, USA) was prepared and diluted with methanol (J.T. Baker 8402, Avantor, Radnor, PA, USA) and filtered (0.22 µm) before injection. Injection volume: 10 µL; flow: 1.0 mL/min; column temperature: 30 °C; detection wavelength: 355 nm. Eluent: 25% of a 0.1% solution of trifluoroacetic acid (TFA, Sigma-Aldrich 302031, St. Louis, MO, USA) in HPLC grade water (*v*/*v*)/75% of a 0.1% solution of TFA in Acetonitrile (J.T. Baker 9017, Avantor, Radnor, PA, USA) (*v*/*v*). Fig. 1 shows a representative HPLC trace.

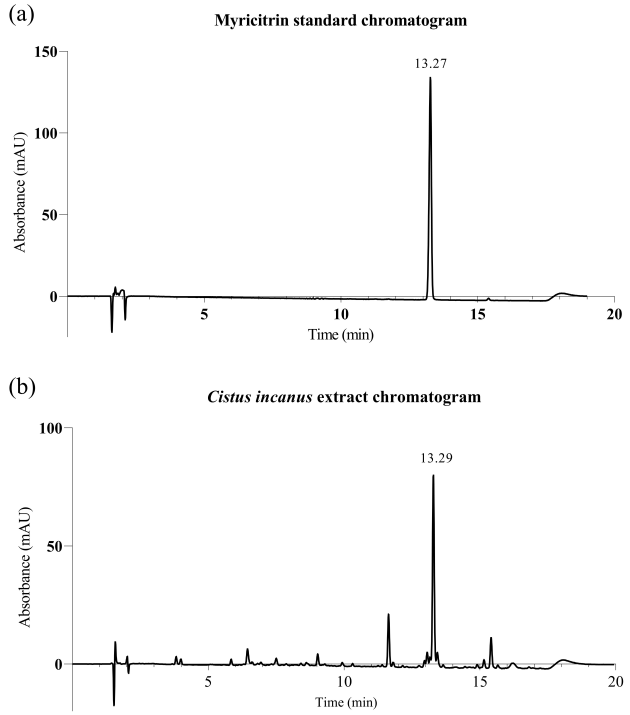


**Fig. 1. High-performance liquid chromatography with diode-array detection (HPLC-DAD) traces for *Cistus incanus* extract.** (a) Myricitrin standard; (b) *Cistus incanus* extract, showing the myricitrin peak (ca. 13.3 min) (detection at 355 nm).

*Trifolium pratense* (Clover) extract (Miniporyl™, IFF – Lucas Meyer Cosmetics, Toulouse, France): aerial parts of flowering red clover were harvested in China (Gansu Province), in full accordance with relevant local regulations. The biomass was extracted in water and ethanol, and the resulting extract was concentrated and spray-dried. After sieving, isopentyldiol was added, and the resulting solution was filtered. The final extract is a transparent liquid, with a pH of 4–8. The extract's content of Biochanin A was titrated by reverse phase HPLC chromatography (Agilent, Santa Clara, CA, USA, 1290 series equipped with Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 1.8 µm column). The *trifolium pratense* extract was diluted at 1/100 in methanol (J.T. Baker 8402, Avantor, Radnor, PA, USA). A Biochanin A standard (Sigma D2016, St Louis, MO, USA) at 1 mg/mL was prepared in methanol. Injection volume: 2 µL; flow: 1.0 mL/min, column temperature: 60 °C, detection wavelength: 260 nm, Eluent: 85% of a 0.1% solution of trifluoroacetic acid (TFA, Sigma-Aldrich 302031, St. Louis, MO, USA) in HPLC grade water (v/v)/and 15% of a 0.1% solution of TFA in Acetonitrile (J.T. Baker 9017, Avantor, Radnor, PA, USA) (v/v). Fig. 2 shows representative HPLC traces.

*Alteromonas* ferment extract: The extremophile *Alteromonas macleodii* (*subsp. fijiensis*), originally isolated from deep-sea hydrothermal vents in proximity to the Galapagos islands, was fermented under conditions propitious to the excretion of a soluble exopolysaccharide (EPS). The ferment was centrifuged and filtered, following which the EPS was precipitated with ethanol, washed, and dried, yielding white cottony fibers. This EPS consists of a repetitive unit of 11 glycosidic residues containing 58% neutral sugars (glucose, galactose, fucose, rhamnose, and mannose) and 30% uronic acids (glucuronic and galacturonic acids). The EPS is finally taken up at 3.0 g/L in water and butylene glycol, resulting in a colorless, slightly viscous aqueous solution, with a pH of 4.70–6.70.

### 2.2.2 Methods

Primary NHEK (Promocell C-12003, Heidelberg, Germany; These cells are tested by the supplier for cell morphology, adherence rate, and cell viability. Immunohistochemical tests for specific cytokeratins are carried out for each lot. Growth performance is tested through multiple passages up to 15 population doublings (PD) under culture conditions without antibiotics or antimycotics. Finally, cells are tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1, HTLV-2, fungi, bacteria, and mycoplasma.) were cultured at 37 °C in keratinocyte basal medium (Promocell C-20216, Heidelberg, Germany) supplemented with 0.06 M $Ca^{2+}$ (Promocell C-34005, Heidelberg, Germany) and a keratinocyte-specific supplement mix (Promocell C-39016, Heidelberg, Germany), under a humidified atmosphere containing 5% $CO_2$. On day 0, cells were seeded so as to reach approximately 60–70% confluence at the time of irradiation.

The tested products were added in culture medium at 0.1% (*Cistus incanus* extract and *Alteromonas* ferment extract) or 0.2% (*Trifolium pratense* extract) on day 1 (D1), 24 h before 5G exposure, and on D2 (just before 5G exposure). The control cultures did not receive any treatment, except for renewal of the culture medium on the same treatment days.

NHEK were exposed to 5G radiation using a Vector Signal Generator MG3710E (Anristsu, Atsugi, Kanagawa, Japan), using the following parameters: frequency: 6 GHz; output power; 15 dBm; irradiance: 20 mW/cm$^2$, for 1 h under a humidified atmosphere containing 5% $CO_2$ (except for an unirradiated, untreated control leg). After irradiation, NHEK from all legs were returned to normal culture conditions (humidified atmosphere containing 5% $CO_2$) for 1 h.

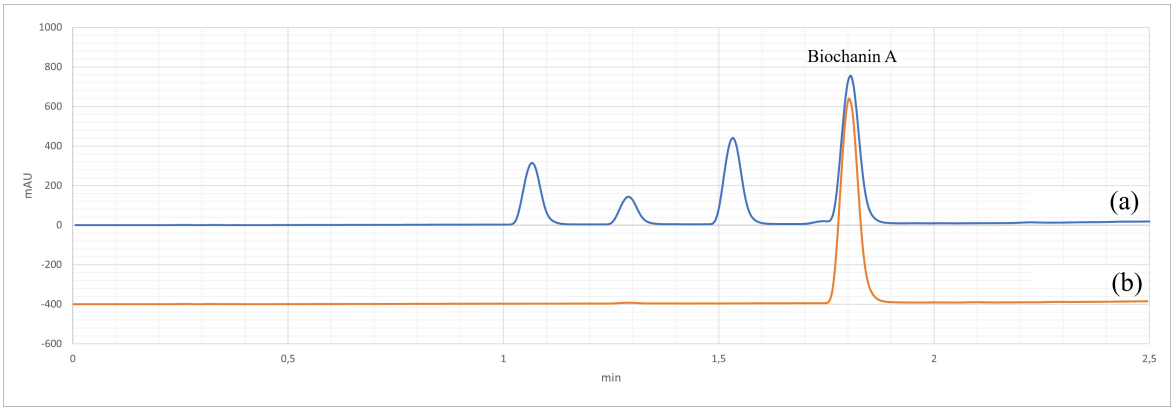


**Fig. 2. HPLC-DAD traces for *Trifolium pratense* extract.** (a) *Trifolium pratense* extract, showing the biochanin A peak (ca. 1.8 min); (b) Biochanin A standard (detection at 260 nm).

As discussed above, the task of defining the most representative set of irradiation parameters is at present extremely complex. The irradiation parameters used in this work were selected to combined high feasibility with general relevance (e.g., frequency and power falling within the generally accepted range of 5G technology).

Culture media were collected and stored at –20 °C pending biochemical assay. IL1-$\alpha$ dosage was performed using a human IL1-$\alpha$ ELISA kit (Cayman Chemical 583301, Ann Arbor, MI, USA). Following the supplier's guidelines, the culture medium and the IL1-$\alpha$ standard were incubated with acetylcholinesterase (AChE) Fab' conjugate, binding IL-1$\alpha$ in wells containing coated IL1-$\alpha$ antibody, overnight at 4 °C. After well plate washing, the reaction product was revealed using an AChE substrate solution. Absorbance was measured at 412 nm using an M200Pro microplate reader (Tecan, Männedorf, Switzerland) and Magellan7 software (Tecan, Männedorf, Switzerland). Six independent biological replicates (6 sampled wells) were included for each set of conditions. The concentration of IL-1$\alpha$ was expressed in pg/mL.

Data analysis was performed using GraphPad Prism 9 (GraphPad Software, Inc., Boston, MA, USA). Statistical tests were selected on the basis of normality and lognormality tests: a parametric test was applied when the normality was positive, and a non-parametric test was used when the normality was negative. For this study, a one-way ANOVA analysis was carried out, followed by Holm-Šídák's multiple comparisons test. Data in the figures is shown as mean $\pm$ Standard Deviation (SD), with *p*-values appearing on brackets.

## 3. Results

### *3.1 Effects of 5G Radiation on an Epidermal Keratinocyte Culture*

When primary Normal Human Epidermal Keratinocytes (NHEK) were exposed to 5G bandwidth radiation, clear effects of 5G radiation on the culture of human epidermal keratinocytes were observable after a remarkably short 1-h exposure time, in several indicators of keratinocyte function (Figs. 3,4), including: a strong reduction in collagenase inhibitor TIMP1; a strong increase in wound healing and epidermal differentiation facilitator ANGPLT4; a marked increase in S100A9, involved in immune recruitment during injury; and, an increase in key inflammatory cytokine IL1-$\alpha$ (whose level was tripled vs. non-irradiated control, albeit with borderline statistical significance).

Interestingly, the effects on all four markers became less marked with longer exposure times (although, generally, some effect was still detectable). The TIMP1 reduction became less intense from 4 h exposure, though it remained noticeable. The increase in ANGPTL4 weakened steadily with increasing exposure time, until at 24 h there was only a slight increase vs. the unexposed control. The increase in S100A9 similarly weakened with increased exposure duration, until there was no noticeable change at 6 h exposure vs. unexposed control, and only a slight change at 24 h exposure. The increase in IL1-$\alpha$ continued to be at least a detectable trend through 6 h exposure, although with lower statistical significance vs. the unexposed control at most time points; however, a steady increase in baseline IL1-$\alpha$ levels was also observed in the unexposed control leg, reaching levels high enough that by 24 h the 5G exposure had essentially no effect (the level in the exposed leg at 24 h was actually somewhat lower vs. the unexposed control, but the difference was not statistically significant). It should be noted that, as culture media were left in continuous contact with the cell culture for the duration of the irradiation, the increase observed in the IL1-$\alpha$ levels likely results at least in part from accumulation into the medium over this period.

### *3.2 Protective Effects of Three Botanical Extracts*

Using the same exposure model, we exposed epidermal keratinocyte cultures to 5G radiation following 24 h pre-treatment with one of several botanical extracts, includ-

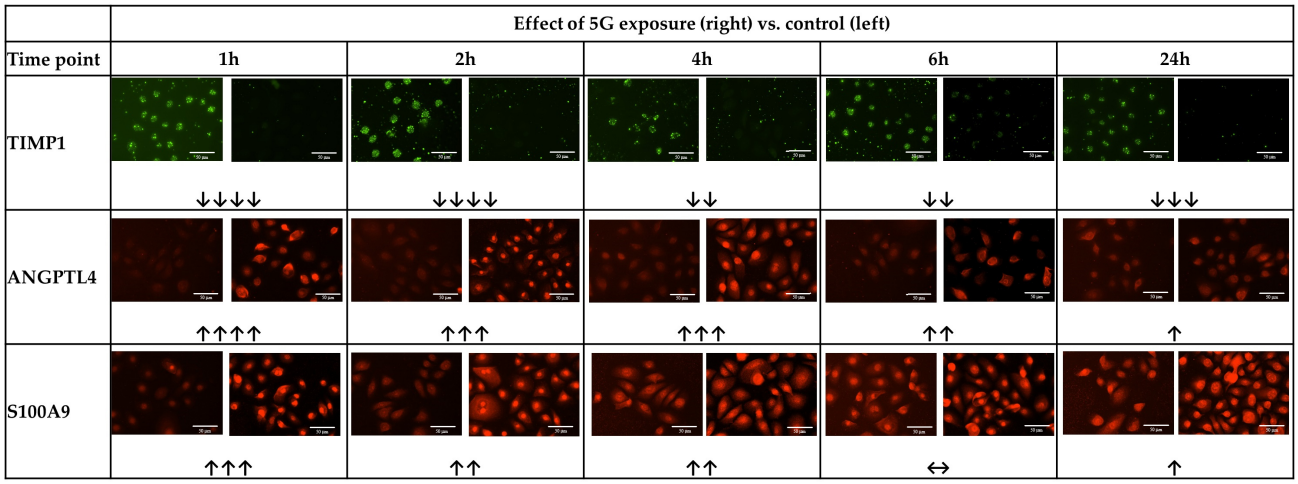


**Fig. 3. Representative images of immunofluorescence staining revealing effects of 5G exposure on keratinocytes.** Typical images of staining for TIMP metallopeptidase inhibitor 1 (TIMP1), Angiopoietin-like protein 4 (ANGPLT4), and S100 calcium-binding protein A9 (S100A9), in a culture of normal human dermal keratinocytes exposed to 5G radiation for 1–24 h. For each timepoint, comparing unirradiated control (Left) with 5G irradiation for the time indicated (Right). Scale bar = 50 µm. Trend evaluation (visual) symbol key: Decrease: ↓↓↓↓: clear; ↓↓↓: fairly clear; ↓↓: moderate; Increase: ↑↑↑↑: clear; ↑↑↑: fairly clear; ↑↑: moderate; ↑: slight. ↔ : no change.

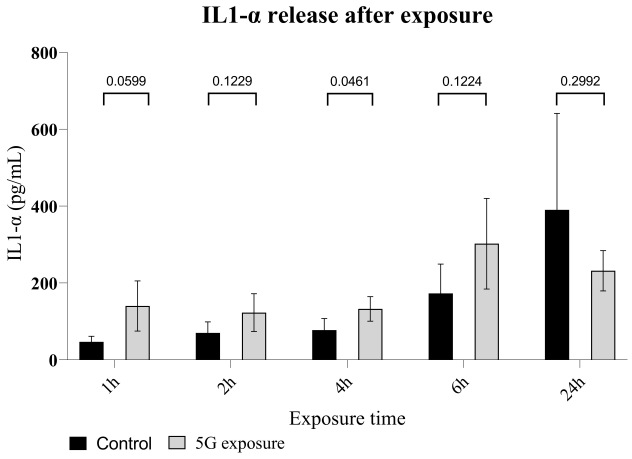


**Fig. 4. Interleukin 1-alpha release in a culture of normal human dermal keratinocytes exposed to 5G radiation.** Enzyme-linked immunosorbent assay (ELISA) measurement of IL1-$\alpha$ after 1–24 h 5G radiation exposure (black: unirradiated control; gray: after irradiation for the time indicated). Data shown as mean +/- SD; brackets indicate *p*-values (unpaired Student *t*-test). Four independent biological replicates (4 sampled wells) were included for each set of conditions.

ing: a *Cistus incanus* extract, a *Trifolium pratense* extract, and an *Alteromonas* ferment extract (in addition to an untreated control leg) (Fig. 5). Here again, after 1h of 5G irradiation, the data show a clear increase in IL1-$\alpha$ release in our keratinocyte culture with irradiation (+68% vs. unirradiated control, from 52.0 to 87.5 pg/mL with $p = 0.00089$). Although the intensity of this increase is less than that observed in the initial study described above, this result still shows an influence of the 5G radiation on the cell culture (and, its statistical significance is actually clearer). On the other hand, we also observe clear protective effects for the three botanical extracts. Two of the three extracts reduce IL1-$\alpha$ release to levels below that of the unirradiated baseline, most strongly so in the case of the *Cistus incanus* extract, which almost totally extinguishes IL1-$\alpha$ release (to 6.3 pg/mL, or –93% vs. irradiated, untreated control, with $p = 0.00000002$; and –88% vs. unirradiated baseline, $p = 0.00021$) (Fig. 5). The *Trifolium pratense* extract reduced IL1-$\alpha$ to 31.7 pg/mL (–64% vs. irradiated control, with $p = 0.00001$; though this level is still somewhat below that of the unirradiated control, the difference does not reach full statistical significance, with $p = 0.07858$). Finally, the *Alteromonas* ferment extract reduced IL1-$\alpha$ to 48.7 pg/mL (–44% vs. irradiated control, with $p = 00071$), returning IL1-$\alpha$ levels to the vicinity of the unirradiated baseline (statistically identical levels, with $p = 0.72338$).

## 4. Discussion

### *4.1 Effects of 5G Radiation on an Epidermal Keratinocyte Culture*

Although the results presented above (especially the results of the immunofluorescence stainings) are mostly qualitative indications, it appears that, after a short exposure time to 5G radiation (1 h), a (negative) influence of this radiation on the exposed epidermal keratinocyte culture can be observed. This includes indications of elevated inflammation status (IL1-$\alpha$), and the activation of homeostasis and damage/injury response mechanisms. A reduc-

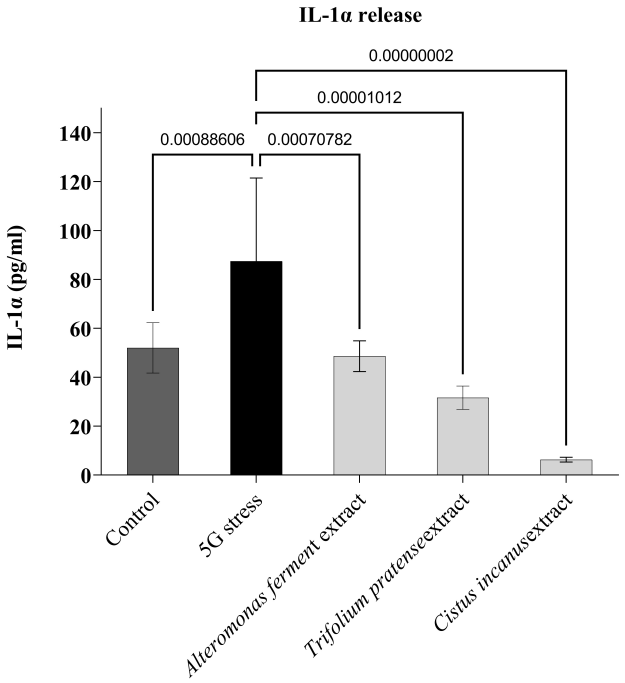


**Fig. 5. Protective effect of three botanical extracts.** IL1-$\alpha$ measurement by ELISA on a culture of normal human dermal keratinocytes exposed to 5G radiation for 1h, as compared to untreated, unirradiated and untreated, irradiated controls. Data shown as mean $\pm$ SD; brackets indicate *p*-values (one-way ANOVA followed by Holm-Šídák's multiple comparisons test). Six independent biological replicates (6 sampled wells) were included for each set of conditions.

tion in collagenase inhibitor TIMP1, for one, may lead to increased collagenase activity in the exposed tissue, leading to extracellular matrix protein degradation and/or its eventual replacement with freshly synthesized material. Increased ANGPLT4, given its role in wound healing and epidermal differentiation, may be symptomatic of the activation of homeostasis mechanisms aiming to repair injury caused by the 5G radiation exposure. The observed increase in S100A9, involved in immune recruitment during injury, would seem to support the same indication. These results are in accordance with the implications of the literature discussed above.

The effects observed with longer, continuous exposure times are somewhat more ambiguous. Indeed, with continued exposure the effects described above seemed to weaken considerably—although in most cases they did not disappear entirely. One could suggest as a possible explanation for these effects the idea that, beyond a relatively short initial shock, represented by the data collected after 1 h 5G radiation exposure, we in fact observing injury response adaptive mechanisms coming into action to mitigate the damage resulting from this exposure, and seek to restore homeostasis. It cannot, of course, be ruled out that other effects may also be in play; the observed increase in baseline IL1-$\alpha$ in the control cultures, for example, may simply be due to accumulation in the medium (left continuously in contact with the cell culture over the duration of exposure), or it may also hint at aging effects in the culture itself—in which case the weakening in the effects observed with 5G irradiation may actually reflect a reduced response and repair ability on the part of the cell culture.

In follow-up studies, it could be very valuable to confirm these directions by increasing exposure times beyond the 24-h mark, with the aim of elucidating whether the cell culture is indeed capable of mitigating the damage caused beyond an initial short-term shock. Further, should this turn out to be the case, a study examining the effect of repeated short-term exposures, seeking to determine whether this type of exposure results in accumulated damage (or, conversely, whether homeostasis mechanisms come into play here also), could be of very high relevance to the continued investigation of the real-world effects of chronic exposure of the population to 5G emissions. Within the bounds of possibility, these studies should use quantitative measures of the effects observed. Finally, additional irradiation parameters such as frequency, intensity, and related others, should be investigated so as to expand the relevance and applicability of this work.

It should be noted that mechanisms such as inflammation and protease activation, both of which our results to be possibly activated by 5G radiation exposure, are widely recognized to lead to premature skin aging, notably via extracellular matrix (ECM) degradation [48]. It thus seems possible that extended and/or chronic exposure to 5G radiation may lead to the premature appearance or worsening of signs of skin aging such as wrinkles, loss of elasticity, pigmentation irregularities, and more.

### *4.2 Protective Effects of Three Botanical Extracts*

As shown above, when we pre-treated our keratinocyte culture with each one of the three botanical extracts presented here, we observed a significantly reduced increase in IL1-$\alpha$ following 1 h exposure to 5G radiation. While keeping in mind the preliminary nature of the data (assessment based on the single IL1-$\alpha$ marker, imperfect reproducibility between studies), and while the specificity of this effect to mitigation of 5G-induced inflammation (vs. general anti-inflammation effects) is as yet unclear, it still appears possible that these results point at least in part to a mitigation of the inflammatory processes triggered by this radiation exposure, and thus indicate that these extracts may have significant potential to ameliorate the effects of 5G radiation on epidermal keratinocytes.

The effects of EM/radiofrequency radiation are largely recognized as proceeding at least in significant part via the increase of oxidative stress (possibly through the generation of Reactive Oxidative Species (ROS)) and the triggering of inflammatory states in living tissue [8,49]. In our own work, as described above, 5G irradiation seems

to trigger inflammatory processes in the keratinocyte culture (as evidenced by increased IL1-$\alpha$). In this light, it could be expected that antioxidant and anti-inflammatory substances may offer some protection against the negative effects of 5G exposure. At present we are only aware of a single directly comparable study in the scientific literature, by Kappler *et al*. [50], demonstrating the protective effects of botanical extracts (in this case, an unspecified extract containing tannins, complex polyphenolic compounds with recognized anti- oxidative properties) against the effects of 5G/radiofrequency irradiation on skin. It is worth noting that the protective effects described by Kappler *et al*. [50] seem to rest mainly on anti-inflammatory and/or anti-oxidant effects (evaluated in their work via effects on IL1-$\beta$ and ROS production, respectively).

The protective effects observed here may thus be considered quite logical given some of the extracts' known composition and biological properties. The *Cistus incanus* extract, which showed the strongest protective effect in our work, has been shown to possess strong anti-inflammatory effects, mediated at least in part via NF-kB [38]. *Cistus incanus* extracts have also been shown to contain polyphenolic compounds (including myricitrin) and possess antioxidant properties [39]. The latter have also been shown *in vivo* with aqueous extracts of the aerial parts of this plant, possibly attributable to their high polyphenol content [51]. It seems likely that antioxidant properties also participate in the observed protective effect. The same logic applies to the *Trifolium pratense* flower extract, which possesses documented antioxidant and anti-inflammatory properties, due at least in part to the presence of flavonoids such as biochanin A [40–53]. For its part, the *Alteromonas* ferment extract has been shown to enhance keratinocyte renewal *in-vitro*, and while the exact mechanisms in play have not been completely elucidated, it is possible that they are linked, directly or indirectly, to the effect we observe here [47]. Some *Alteromonas* ferment extracts have also been shown to possess antioxidant and anti-inflammatory properties [52,53]. Finally, it is notable that $\beta$-glucan polysaccharides have been reported as showing protective effects against oxidative injury caused by EM radiation at 2.45 GHz (bordering on the energy ranges relevant to 5G) in rat skin tissue [54].

The specificity of the effect of the extracts tested here is not yet clear, and may vary from extract to extract. In the case of the *Cistus incanus* extract, which reduced IL1-$\alpha$ production to levels well below those of the unirradiated control leg, it is likely that the effect extends to more than just countering the effects of the irradiation itself, and at least part of the efficacy observed is linked to the extract's known anti-inflammatory effects (discussed above). Treatment with the other two extracts returned IL1-$\alpha$ levels to the vicinity of that observed in the unirradiated control leg—leaving open the possibility that in those cases the effect is more specific to IL1-$\alpha$ production induced by the irradiation (though of course this does not definitely prove this point on its own, and we could also be observing a weaker, nonspecific effect). It would likely be valuable to include treated, un-irradiated cultures in future studies, in an attempt to clarify this point.

In follow-up studies, it would likely be advisable to improve the repeatability of this model, seeking to overcome some of the variability observed between our two studies. The consistency of the primary cells used in the cultures could be a relevant area of focus, for instance—or conversely, it could be interesting to explore the effects of this irradiation on cells from different provenances (e.g., covering a range of donor ages, among other individual characteristics which could influence the results), as well as on additional skin cell types. It may also be valuable to explore the effect of these extracts, and possibly others, on other processes and biological markers which have been shown, here and elsewhere, to be impacted by exposure to 5G radiation. Also, as noted above, it would be interesting to evaluate the protective effect of these and other botanical extracts on a keratinocyte culture exposed to 5G radiation for longer durations (up to, and beyond, 24 h), as well as for short but repeated durations. Additional irradiation parameters such as frequency and intensity should be varied in the context of treatment with botanical extracts, in order to demonstrate protective effects over a wider range of real-life conditions. As suggested by Kostoff *et al*. [17], it may be useful to examine signal pulsation and modulation, to more closely mimic actual exposure.

## 5. Conclusions

The results presented above represent a positive indication as to the potential value of this preliminary cell-based model as a tool for the identification of substances capable of protecting human skin tissue from the effects of 5G radiation, as well as for the *in-vitro* evaluation of these same effects and the mechanisms involved.

Our data indicate a clear influence of 5G irradiation on the keratinocytes, suggesting induced changes in skin homeostasis which may be consistent with a state of injury and damage response. This appears to corroborate published findings as discussed above, and supports the notion that 5G irradiation may have deleterious effects on the health, and therefore also the appearance, of human skin—possibly, with extended chronic use, leading to premature skin aging and/or the early appearance or worsening of aging signs such as wrinkles or pigmentation irregularities.

Most importantly for the ultimate purposes of this work, the results discussed above demonstrate the potential value of three botanical extracts (extracts of *Cistus incanus* aerial parts; *Trifolium pratense* flowers; and *Alteromonas* ferment) as active ingredients in topical formulations intended to offer protection of human skin from 5G radiation. Best results were obtained with the *Cistus incanus* extract, likely proceeding via anti-inflammatory and/or anti-oxidant mechanisms.

In future studies, it may be possible to explore these effects in more depth, exploring both the mechanisms involved in the effects of 5G radiation on human skin and the protective effects to be obtained from the use of these and other botanical extracts, with the goal of providing increasingly effective solutions for daily users of 5G technology.

## Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Author Contributions

MC, SK, FH, and JA conceptualized and designed the studies presented here; MC and FH were responsible for study execution and data acquisition; MC performed data analysis, curation, and visualization; FH and MC were responsible for data interpretation; JA provided funding and resources, and was responsible for overall project supervision; FH wrote the manuscript, and FH, SK, MC, and JA contributed to its review and edition. All authors have read and approved the present manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

## Ethics Approval and Consent to Participate

Aerial parts of *Trifolium pratense* and *Cistus incanus* were harvested in full observance of respective local regulations, by or on behalf of IFF Ltd. - Lucas Meyer Cosmetics. Not relevant for *Alteromonas*, produced as a microbial culture.


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## Conflict of Interest

The authors are employees of International Flavors and Fragrances and its affiliates, and declare no conflict of interest. Beyond funding our work, the company as such had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing or editing of the manuscript; or in the decision to publish the results.

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