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CAPSICUM ANNUUM L EXTRACTS MODULATE CELL
PROLIFERATION AND INTRACELLULAR Ca^{2+}
SIGNALING IN HUMAN MAMMARY EPITHELIAL CELLS

EXTRACTOS DE *CAPSICUM ANNUUM* L MODULAN LA
PROLIFERACIÓN CELULAR Y LA SEÑALIZACIÓN
INTRACELULAR DE Ca^{2+} EN CÉLULAS
EPITELIALES MAMARIAS HUMANAS



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***CAPSICUM ANNUUM* L EXTRACTS MODULATE CELL PROLIFERATION AND INTRACELLULAR Ca^{2+} SIGNALING IN HUMAN MAMMARY EPITHELIAL CELLS**

EXTRACTOS DE *CAPSICUM ANNUUM* L MODULAN LA PROLIFERACIÓN CELULAR Y LA SEÑALIZACIÓN INTRACELULAR DE Ca^{2+} EN CÉLULAS EPITELIALES MAMARIAS HUMANAS

ABSTRACT

This study evaluated the effect of extracts from three varieties of *Capsicum annuum* L on cellular metabolic activity and intracellular Ca^{2+} signaling in human breast epithelial cells, using a tumor cell line (MCF-7) and a non-tumor cell line (MCF-12F). Metabolic activity was determined using the Alamar Blue assay, while Ca^{2+} dynamics were analyzed using the Fluo-4 AM fluorescent indicator and real-time functional microscopy. The plant extracts produced differential effects depending on cell type and fruit variety. More pronounced changes in metabolic activity were observed in MCF-7 cells, while in MCF-12F cells, transient increases in intracellular Ca^{2+} were recorded with distinct kinetic patterns among varieties. The results indicated that complex phytochemical matrices derived from *Capsicum annuum* L interact with the Ca^{2+} signaling machinery and can modify cellular physiology in a tumor-context-dependent manner. These findings provide experimental evidence regarding the interaction between plant-derived compounds and fundamental cellular pathways related to human health.

Keywords: capsicum annuum L, intracellular calcium signaling, human mammary cells, cell proliferation, functional foods

RESUMEN

El presente estudio evaluó el efecto de extractos de tres variedades de *Capsicum annuum* L sobre la actividad metabólica celular y la señalización intracelular de Ca^{2+} en células epiteliales mamarias humanas, utilizando una línea tumoral (MCF-7) y una no tumoral (MCF-12F). La actividad metabólica se determinó mediante el ensayo Alamar Blue, mientras que la dinámica de Ca^{2+} se analizó utilizando el indicador fluorescente Fluo-4 AM y microscopía funcional en tiempo real. Los extractos vegetales produjeron efectos diferenciales dependiendo del tipo celular y de la variedad del fruto. En células MCF-7 se observaron cambios más pronunciados en la actividad metabólica, mientras que en MCF-12F se registraron incrementos transitorios de Ca^{2+} intracelular con patrones cinéticos distintos entre variedades. Los resultados indicaron que matrices fitoquímicas complejas derivadas de *Capsicum annuum* L interactúan con la maquinaria de señalización de Ca^{2+} y pueden modificar la fisiología celular de manera dependiente del contexto tumoral. Estos hallazgos aportan evidencia experimental sobre la interacción entre compuestos de origen vegetal y rutas celulares fundamentales relacionadas con la salud humana.

Palabras clave: capsicum annuum L, señalización de calcio intracelular, células mamarias humanas, proliferación celular, alimentos funcionales

1. INTRODUCTION

In vitro studies generate testable biological hypotheses. This knowledge enables actions to be taken that improve the quality of life of organisms. In this way, our understanding is enriched, and we gain insight into the physiological complexity of organisms. This study reviewed information on ionized calcium (Ca^{2+}), a fundamental cation in the cells of all organisms. Changes in intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) promote its activity as a second messenger. Essentially, changes in its concentration translate extracellular stimuli into specific intracellular responses, such as proliferation, secretion, differentiation, and programmed cell death.

To successfully carry out the multitude of cellular processes, the coordinated participation of proteins with highly specific functions is required; these proteins are located in various organelles and membranes. Essentially, these proteins are coupled to the Ca^{2+} gradient and maintain its intracellular concentration at around 100 nanometers (nM). Therefore, Ca^{2+} is transported out of the cell or stored in organelles. The main Ca^{2+} -sensitive molecular entities are voltage-dependent and voltage-independent Ca^{2+} channels; the transient receptor potential (TRP); plasma membrane Ca^{2+} pumps (PMCA); endoplasmic reticulum Ca^{2+} pumps (SERCA); the activity of intracellular Ca^{2+} channels such as the ryanodine receptor (RyR); and the inositol trisphosphate receptors (IP_3R), among many others. As mentioned earlier, intracellular calcium signaling regulates essential cellular processes such as proliferation, metabolism, and cell survival, and its dysregulation is a hallmark of breast cancer (Berridge, 2016; Prevarskaya et al., 2018; Monteith et al., 2017).

To date, it is known that 10–15% of breast cancer cases are hereditary. Therefore, most cases are caused by risk factors that can be modified through lifestyle changes, including dietary habits (excessive intake of fats, sugars, red meat, and alcohol, among the main ones). Other factors include age, ethnicity, hormonal status, ultraviolet radiation, and disruptions in circadian rhythms (changes in sleep-wake cycles). However, diet is a factor that can be controlled with relative ease, and in this regard, observational and epidemiological studies have suggested the positive potential of phenolic compounds in chemoprevention and chemosensitization for breast cancer, as they have been attributed protective and anticancer effects (Ávila-Gálvez et al., 2020).

Furthermore, dietary intake of carotenoids has been shown to be beneficial to human health, as it protects the body against chronic diseases, including cancer. This is partly due to their antioxidant activity, which can modulate DNA repair, cell proliferation, apoptosis induction, and angiogenesis inhibition (Dehnavi et al., 2024). These effects result from the modulation of signaling pathways, gene expression, and protein expression through interactions with pathways that influence cell viability, proliferation, differentiation, and invasion. However, further studies are still needed to support their use in humans (Ávila-Gálvez et al., 2020).

The fruits of *Capsicum annum* L contain various bioactive phytochemicals capable of interacting with key cellular pathways (Materska & Perucka, 2005; Zimmer et al., 2012; Wahyuni et al., 2013). Unlike studies based on

isolated compounds, the analysis of plant extracts allows for the evaluation of synergistic interactions among multiple secondary metabolites, which more accurately reflects real-world dietary exposure conditions. Therefore, the objective of this study was to evaluate the effect of extracts from three varieties of *Capsicum annuum* L var. Fascinatum (FAS), Baselga (BAS), and Orangelita (ORA)— on metabolic activity and intracellular Ca²⁺ signaling in human mammary epithelial cell lines, one cancerous (MCF-7) and one non-tumorous (MCF-12F).

2. METHOD OF RESEARCH

Metabolic activity was determined using the Alamar *Blue* assay and Ca²⁺ dynamics. The analysis was performed using the Fluo-4 AM fluorescent indicator and real-time functional microscopy. The extracts evaluated differentially modulated cell proliferation depending on cell type and fruit variety. In MCF-7 cells, more pronounced changes in metabolic activity were observed, whereas in MCF-12F cells, the extracts induced transient increases in intracellular Ca²⁺ with distinct kinetic patterns among varieties.

2.1. Cell culture

The human epithelial cell lines MCF-7 and MCF-12F were donated by Dr. Aceves and Dr. Delgado (Institute of Neurobiology, National Autonomous University of Mexico [UNAM]); they were originally obtained from the *American Type Culture Collection* (ATCC). Both lines were maintained in *Dulbecco's Modified Eagle Medium* ([DMEM] supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin), following standard protocols for human breast cell culture (Freshney, 2016; Lacroix & Leclercq, 2004). The cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO₂.

Cell expansion was performed using healthy, confluent cultures. For subculturing, the monolayers were incubated for 30 seconds with Verseno, followed by a 60-second incubation with 0.5% trypsin, in accordance with widely used procedures for cell dissociation (Freshney, 2016). The action of trypsin was inactivated by adding complete DMEM medium. Subsequently, the cells were dissociated and seeded into 60-mm culture dishes at a density of 1 × 10⁶ cells for proliferation. All experiments were performed using cultures 18 to 24 hours after trypsinization, in order to control the degree of cell confluence and minimize variations associated with the cell cycle (Lacroix & Leclercq, 2004).

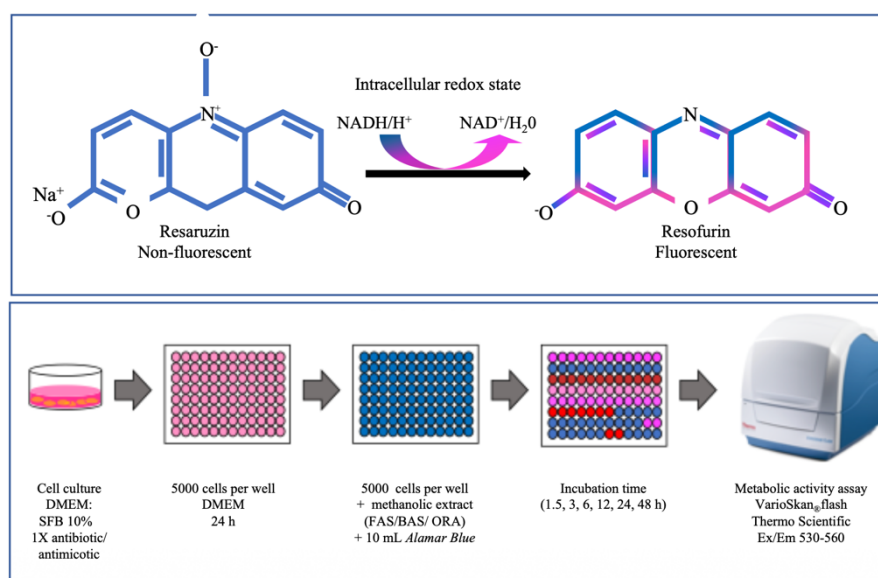
2.2. Assessment of cell proliferation

Cell viability and metabolic activity following exposure to BAS, ORA, and FAS extracts were assessed using the *Alamar Blue* (AB; *Thermo Fisher Scientific*), a widely validated method for measuring cellular metabolic activity and *in vitro* cytotoxicity (Rampersad, 2012; O'Brien et al., 2000). This assay is based on the reduction of resazurin to resorufin, a highly fluorescent compound, which allows for indirect quantification of cellular metabolic status without inducing lysis, enabling kinetic measurements over time. Cells were seeded in 96-well plates at a density of

0.05×10^6 cells per well. Subsequently, the extracts were added at different concentrations (0.1, 1, and 10%), and metabolic activity was assessed at 0, 1.5, 3, 6, 12, 24, 48, and 72 hours (Figure 1). Alamar Blue reagent was added to the medium at a final concentration of 10% (v/v). Fluorescence was measured using a multimodal plate reader (Varioskan[™] Lux; Thermo Fisher Scientific) at excitation wavelengths of 530-560/590 nm (O'Brien et al., 2000).

Figure 1

Schematic diagram of the Alamar Blue assay principle used to assess cellular metabolic activity through the reduction of resazurin to fluorescent resorufin

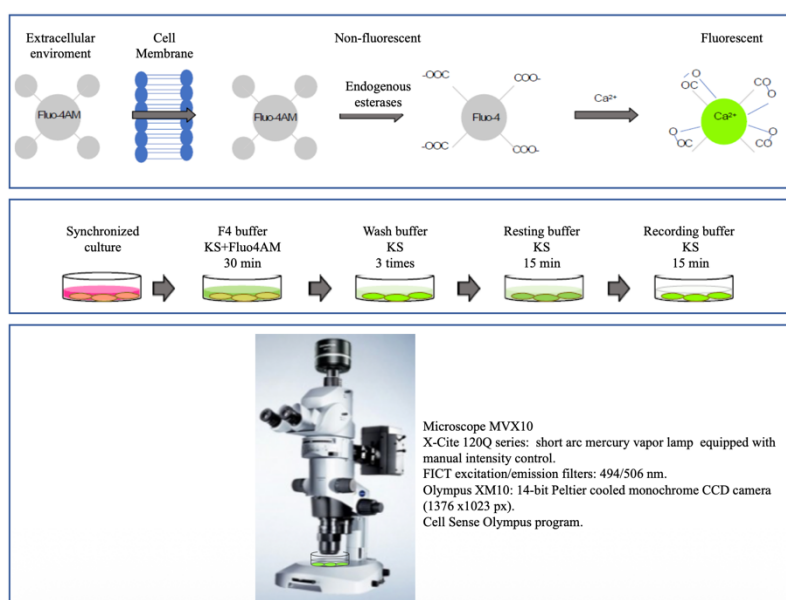


2.3. Determination of intracellular Ca²⁺

Changes in intracellular Ca²⁺ concentrations induced by stimulation with *Capsicum annuum* L extracts were assessed using the fluorescent indicator Fluo-4 AM, in accordance with previously established methodologies (Gee et al., 2000; Paredes et al., 2008). Fluo-4 AM exhibited high sensitivity to intracellular Ca²⁺, with a significant increase in fluorescence upon binding to the ion, and is widely used for studying Ca²⁺ signaling in living cells. The cells were incubated for 30 minutes with Krebs-F4 solution (in mM: NaCl 150, KCl 1, MgCl₂ 1.8, CaCl₂ 1.8, Glucose 4, HEPES 10, pH 7.4; supplemented with bovine serum albumin, pluronic acid, and 5 μM Fluo-4 AM). Subsequently, the preparations were washed with Krebs solution and incubated for an additional 10–15 min to allow for complete removal of the indicator prior to fluorescence recording (Figure 2) (Saldaña et al., 2009).

Figure 2

Cell loading with the fluorescent probe Fluo-4 AM for the detection of changes in intracellular Ca^{2+} concentration in human mammary epithelial cells



2.4. Functional microscopy

The images were acquired using an MVX10 microscope equipped with a 20× objective, at a rate of four images per second. Fluo-4 excitation and emission were set at 494/506 nm, respectively, following standard protocols for functional Ca^{2+} microscopy. To minimize photobleaching, the mercury lamp power was reduced to 5%. For quantitative analysis, 50 cells per field were randomly selected, and grayscale fluorescence values were measured; these were subsequently analyzed using *OriginLab 2021* software, as previously described in studies of intracellular Ca^{2+} dynamics (Saldaña et al., 2009).

3. RESULTS

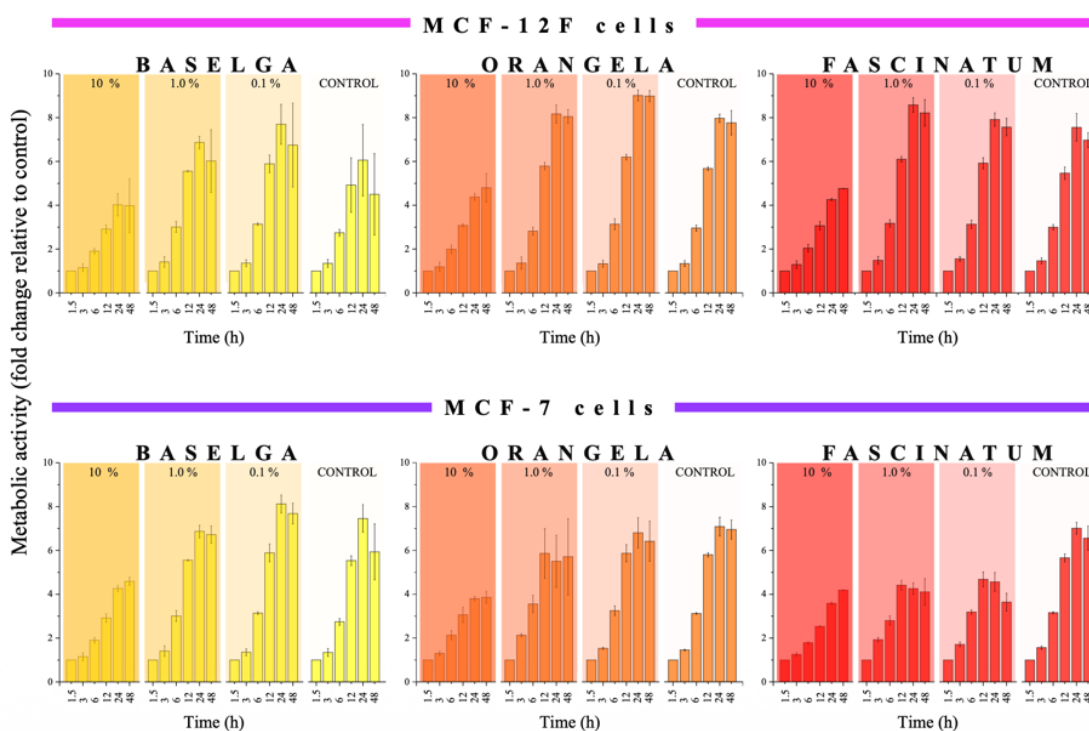
3.1. Cellular metabolic activity following exposure to extracts of *Capsicum annuum* L

Exposure of human breast epithelial cells to extracts of *Capsicum annuum* L produced differential changes in metabolic activity depending on both the cell type and the variety of extract analyzed. MCF-7 cancer cells showed a more pronounced response than non-tumor MCF-12F cells, indicating greater sensitivity of the tumor phenotype to phytochemical compounds.

The dose-dependent metabolic activity curves indicated that, for all three 10% methanolic extracts, both cell lines exhibited a decrease in metabolic activity compared to their respective controls, strongly suggesting that this effect is related to the saturating dose. In fact, at a 1% concentration, the extracts from BAS and ORA showed no changes compared to the control. Although at 0.1% ORA, only the MCF-12F cells showed an increase in metabolic activity, while the MCF-7 cells behaved similarly to the control. Interestingly, the extract from the FAS variety has differential effects on both cell lines at concentrations of 0.1–1%. The results indicated that MCF-7 cells are more sensitive to exposure to this extract (Figure 3).

Figure 3

*Metabolic activity of MCF-7 and MCF-12F cell lines following exposure to extracts from three varieties of *Capsicum annuum* L. Representative experiments are shown.*



Note. Data are expressed as mean \pm SD (n=4). Significant differences: $p < 0.05$.

Taken together, these results indicated that extracts of *Capsicum annuum* L modulate cell proliferation in a context-dependent manner, with tumor cells being particularly susceptible to their action.

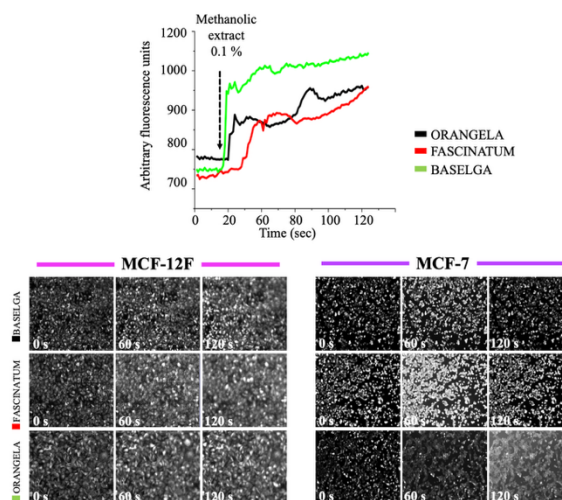
3.2. Dynamics of intracellular Ca²⁺ signaling

To assess whether the observed metabolic changes were associated with alterations in intracellular signaling, changes in cytosolic Ca²⁺ concentration were analyzed. Extracts of *Capsicum annuum* L induced dynamic intracellular Ca²⁺ responses characterized by transient increases in fluorescence.

The BAS extract produced a rapid and sustained increase in intracellular Ca²⁺ during the first few seconds following stimulation. In contrast, the ORA and FAS extracts produced more gradual increases of smaller amplitude. These differences suggest the differential activation of cellular mechanisms responsible for the influx, release, or reuptake of Ca²⁺ (Figure 4).

Figure 4

*Temporal dynamics of intracellular Ca²⁺ signaling induced by extracts of *Capsicum annuum* L in human mammary epithelial cells*



The observed kinetic variations indicated that the phytochemical composition of each plant variety influences how intracellular signaling is modulated, including in non-cancerous cells.

3.3. Visual evidence of intracellular Ca²⁺ mobilization

Fluorescence images obtained before and after stimulation confirmed the intracellular mobilization of the ion. Following the application of the extracts, a clear increase in cytosolic fluorescence was observed in the treated cells, consistent with the previously recorded rise in intracellular Ca²⁺. The spatial distribution of the signal suggests

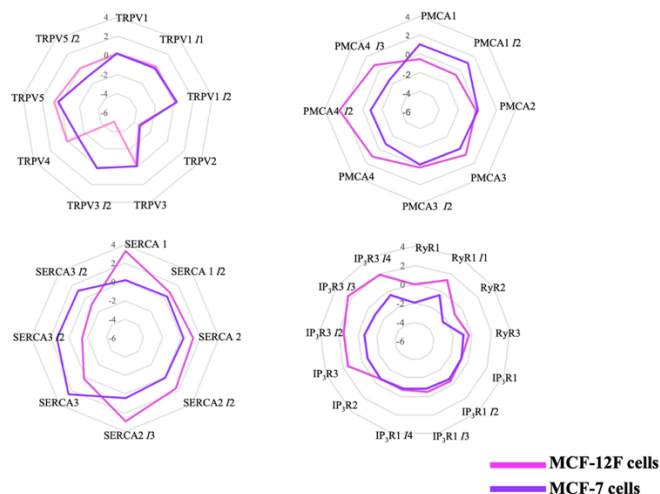
the involvement of intracellular reservoirs in the generation of the observed responses, which is consistent with the dynamic nature of Ca^{2+} as a second messenger (Figure 5).

3.4. Gene expression profile associated with Ca^{2+} homeostasis

Comparative gene expression analysis revealed notable differences between the cell lines studied in genes related to Ca^{2+} homeostasis. MCF-7 cells exhibited a different expression pattern than MCF-12F cells in families of regulatory proteins such as TRP, RyR, and IP_3R channels, as well as SERCA and PMCA pumps.

These variations suggest that the molecular machinery responsible for intracellular Ca^{2+} regulation is altered in the tumor phenotype, which could explain the greater magnitude of the functional responses observed following exposure to plant extracts (Figure 5). Taken together, the functional results and bioinformatic analysis indicated that the cellular response to *Capsicum annuum* L extracts depended not only on phytochemical composition but also on the cells' prior molecular state.

Figure 5
Gene expression profile associated with Ca^{2+} homeostasis



Note. MCF-7 cells (purple) and MCF-12F cells (pink).

4. DISCUSSION

The results demonstrated that extracts of *Capsicum annuum* L are capable of simultaneously modulating cellular metabolic activity and intracellular Ca^{2+} signaling in human mammary epithelial cells. The magnitude and nature of the response depended on both the plant variety and the cell type, suggesting that the interaction between complex phytochemical matrices and cellular physiology is highly context-dependent.

The greater sensitivity observed in MCF-7 cells compared to MCF-12F cells indicated that the tumor phenotype exhibited differential susceptibility to the action of bioactive compounds of plant origin. This behavior can be explained by the metabolic reprogramming characteristic of cancer cells, which involves modifications in intracellular signaling pathways, particularly those dependent on Ca²⁺ (Monteith et al., 2017; Prevarskaya et al., 2018). In this context, small variations in ion homeostasis can result in significant changes in cell proliferation and viability.

The transient increases in Ca²⁺ induced by the extracts suggest the activation of early signaling mechanisms rather than a direct cytotoxic effect. Ca²⁺ acts as a universal second messenger, integrating external stimuli with cellular responses such as proliferation, differentiation, or programmed cell death (Clapham, 2007; Berridge, 2016). Therefore, the modulation of its intracellular dynamics constitutes a critical point through which dietary compounds can influence cellular behavior.

The kinetic differences between plant varieties indicated that the effect is not due to a single molecule, but rather to the combined action of multiple phytochemicals present in the food matrix (Chen et al., 2021). This finding was significant because much of the biomedical research has focused on isolated compounds, whereas under real-world consumption conditions, organisms are exposed to complex mixtures. The evidence obtained supports the hypothesis that functional foods exert their biological effects through the modulation of cellular signaling systems and not solely through general antioxidant actions.

The differential gene expression profile associated with Ca²⁺ regulatory proteins supported this interpretation. The remodeling of the intracellular ion transport machinery in tumor cells could explain the greater amplitude of response observed following stimulation with the extracts (Zhai et al., 2020). Consequently, the cellular response does not depend exclusively on the chemical composition of the food, but also on the cell's prior physiological state.

Overall, the results suggest that *Capsicum annuum* L may influence fundamental cellular processes by modulating Ca²⁺ signaling. This effect opens up the possibility of considering plant-based foods not only as nutritional sources but also as modulators of cellular pathways relevant to human health. Although the present study was limited to an *in vitro* model, it provided experimental evidence that contributes to understanding the cellular mechanisms that could link diet and chronic degenerative diseases. A limitation of the present study was the exclusive use of *in vitro* cellular models; therefore, physiological extrapolation requires validation in more complex biological systems.

Future studies should identify the responsible compounds and evaluate their effects in more complex physiological models. However, the findings presented here establish a mechanistic basis for investigating the role

of phytochemical-rich foods in preventive and public health strategies aimed at modulating cellular processes associated with human diseases..

5. CONCLUSIONS

Extracts of *Capsicum annuum* L modulate metabolic activity and intracellular Ca²⁺ signaling in human breast epithelial cells, showing differential responses between tumor and non-tumor cells. The observed changes indicate that complex phytochemical matrices can interact with fundamental cellular pathways associated with proliferation and ionic homeostasis.

The findings provided experimental evidence that compounds found in plant-based foods can act as modulators of cellular processes relevant to health. This study provided a mechanistic basis for exploring the relationship between diet, cellular signaling, and the potential role of phytochemical-rich foods in modulating cellular processes associated with chronic degenerative diseases.

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