

Article

Dual Bioactivity of Curcuminoid Extract from *Curcuma longa*: Acaricidal Efficacy Against Dust Mites and Anti-Inflammatory Potential in Immune and Skin Cells

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Abstract

House dust mites (HDMs) are major indoor allergens linked to asthma and atopic dermatitis, creating demand for safer, natural alternatives to synthetic acaricides and anti-allergy agents. This study aimed to investigate the dual bioactivity of curcuminoid extract from *Curcuma longa* to address HDM allergy through two distinct but complementary scopes: an environmental approach evaluating direct acaricidal efficacy to reduce allergen exposure and a cellular approach investigating the anti-inflammatory potential to mitigate the host's underlying immune response. Curcuminoids were extracted using Soxhlet extraction with 70% ethanol and characterized via TLC, FTIR-ATR, and LC-MS. Acaricidal activity against HDMs was evaluated using extract-impregnated filter papers (0.1–10 mg/mL) over 24 h. Cytotoxicity and anti-inflammatory effects were assessed in HaCaT keratinocytes and RAW264.7 macrophages through cell viability, migration, phagocytic activity, nitric oxide (NO) inhibition, and gene expression assays. The curcuminoid extract demonstrated potent, dose-dependent acaricidal activity against HDM, achieving 100% mortality at 10 mg/mL within 24 h. A safe sub-cytotoxic concentration ($\leq 0.001 \mu\text{g}/\mu\text{L}$) preserved HaCaT cell migration and metabolic activity. In LPS-stimulated RAW264.7 cells, curcuminoids significantly reduced inflammatory NO production ($p < 0.01$) while enhancing phagocytic activity. Overall, these findings highlight curcuminoids as a sustainable natural strategy for reducing HDM exposure and mitigating allergic inflammation.

Keywords: curcuminoids; asthma; dust mites; toxicity assay; anti-inflammatory



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1. Introduction

House dust mites (HDMs), particularly *Dermatophagoides pteronyssinus* and *D. farinae*, are among the most significant sources of indoor allergens worldwide and a major cause of allergic rhinitis, atopic dermatitis, and asthma exacerbations [1,2]. These microscopic arthropods thrive in household environments and produce potent allergens capable of triggering hypersensitivity reactions in genetically predisposed individuals. More than 50% of allergic patients are sensitized to HDM allergens, highlighting their public health relevance [2].

Conventional management strategies for HDM-related allergic diseases often rely on allergen avoidance, pharmacotherapy, and immunotherapy. However, these approaches can be limited by incomplete efficacy, side effects, and cost. Therefore, there is increasing interest in eco-friendly, naturally derived agents that can both reduce allergen load and modulate the underlying inflammatory responses.

Curcuminoids (Figure 1), polyphenolic compounds extracted from the rhizome of *Curcuma longa*, have been used for centuries in traditional medicine for their anti-inflammatory, antioxidant, and antimicrobial properties [3]. In recent years, extensive research has demonstrated curcuminoids' ability to modulate key immune responses, including inhibition of pro-inflammatory cytokines, suppression of immune cell activation, and regulation of signaling pathways such as NF- κ B, ultimately contributing to reduced inflammation and balanced immune function [4–9]. Furthermore, curcumin has exhibited pesticidal and insecticidal activities against agricultural pests and mosquitoes [10–12], suggesting potential acaricidal effects against HDMs.

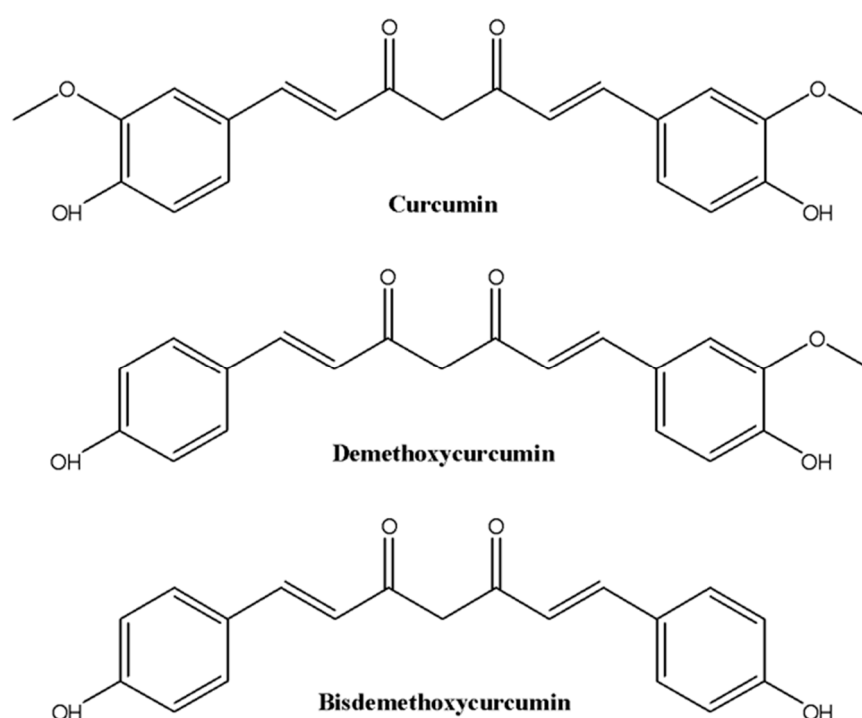


Figure 1. Chemical structures of the major curcuminoids present in *Curcuma longa* extract: curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC).

Despite this evidence, no study to date has comprehensively evaluated the dual role of curcuminoids as both a mite-controlling agent and a modulator of epithelial and immune responses relevant to HDM-associated allergy. To address this gap, the present study investigates the bioactivity of *Curcuma longa* curcuminoid extract with two overarching objectives: (i) to evaluate its acaricidal efficacy against HDMs as an eco-friendly household intervention to reduce allergen exposure and (ii) to determine its immunomodulatory and cytoprotective effects in skin and immune cell models implicated in allergic disease pathogenesis. This study investigates the potential of curcuminoids as a natural acaricide—an agent used to eliminate dust-mite populations—to reduce the indoor allergen load associated with allergic diseases. While the potent capability of using curcuminoids for this purpose has been recognized, real-world application requires further experiments. Specifically, household-level trials are essential to confirm efficacy under actual environ-

mental conditions, as well as the development of advanced delivery systems to overcome challenges related to the compounds' low solubility and stability.

In this study, the safety and biological activity of curcuminoids were systematically evaluated using HaCaT human keratinocytes and RAW264.7 murine macrophages. HaCaT cells are an immortalized human keratinocyte cell line derived from adult skin. They retain the essential characteristics of normal epidermal keratinocytes, including differentiation capacity, barrier-related functions, and responsiveness to inflammatory and allergenic stimuli [13]. The inclusion of the HaCaT skin cell line is particularly important, as it enables direct evaluation of curcuminoids' suitability for topical applications aimed at reducing allergen-induced skin manifestations such as urticaria. Their observed effects on keratinocyte viability, wound healing potential, inflammatory gene regulation, and membrane integrity collectively highlight the capacity of curcuminoids to support skin barrier repair while suppressing inflammatory responses triggered by HDM allergens. RAW264.7 cells are a murine macrophage-like cell line originating from BALB/c mice. They are commonly used as models of innate immune responses because they exhibit key macrophage functions, such as phagocytosis, nitric oxide production, and cytokine secretion, when stimulated by agents such as LPS. This makes RAW264.7 cells suitable for evaluating the anti-inflammatory, immunomodulatory, and antimicrobial activities of natural compounds [14,15]. Hence, these specific cell lines were used to assess the potential of curcuminoids to modulate immunological and inflammatory mechanisms underlying dust-mite-induced allergic responses, enabling evaluation of innate immune functions relevant to HDM-associated allergy.

When considered alongside their demonstrated acaricidal properties, these findings position curcuminoids as compelling dual-action candidates. On one hand, curcuminoids show potential for development into household formulations specifically designed to reduce dust-mite populations, thereby lowering allergen exposure at the environmental level. On the other hand, their immunomodulatory and skin-protective activities highlight their suitability for future topical or oral formulations aimed at alleviating cutaneous and systemic allergic symptoms associated with dust-mite allergens. Collectively, these complementary applications underscore curcuminoids as versatile natural agents capable of addressing both the source of allergen exposure and the resulting allergic manifestations.

2. Materials and Methods

2.1. Chemicals

All of the chemicals used in this study were of analytical grade and purchased from Thermo Fisher Scientific (Waltham, MA, USA) or Sigma-Aldrich (Saint Louis, MO, USA). The following chemicals and reagents were used: Dulbecco's Modified Eagle's Medium (DMEM/F12; nutrient mixture, Ref. D8437), Heat-inactivated fetal bovine serum (Ref. F9665), Penicillin–streptomycin (Ref. P4333), Trypsin-EDTA solution, Thiazolyl blue tetrazolium bromide (Ref. M5655), Dimethyl sulfoxide (DMSO, Ref. 472301), Mitomycin C ready-made solution (Ref. M5353, 10 mg/mL), Lipopolysaccharides (LPS, Ref. L2880, *Escherichia coli* O55:B5), Crystal violet (Ref. C0775), and Nitrite assay kit (Griess Reagent Kit, Catalog Numbers G7921, Invitrogen™, Waltham, MA, USA). Additionally, the following reagents were obtained from Thermo Scientific™ (Bremen, Germany), and used according to the manufacturer's instructions: alamarBlue™ Cell Viability Reagent, Neutral red, SuperScript™ IV CellsDirect™ cDNA Synthesis Kit (Ref. 11750150), and PowerTrack™ SYBR™ Green Master Mix for qPCR (Ref. A46109).

2.2. Collection of Turmeric Samples

Rhizomes of the *Curcuma longa* plant (turmeric) were obtained from selected commercial cultivations in the Kurunegala District (7.4871° N, 80.3649° E), which is considered a main turmeric-growing area in the intermediate zone of Sri Lanka [16]. *Curcuma longa* L. (*Zingiberaceae*) rhizomes were authenticated from the National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka. The rhizomes were air-dried for 3–4 days until they became crisp. The rhizomes were peeled, chopped, and pulverized, followed by sieving through a strainer with a mesh size of 100 µm. The powdered product was used in further experiments.

2.3. Extraction of Curcuminoids from Turmeric

Soxhlet extraction was performed to isolate the active compounds from turmeric powder. A 50 g sample of the turmeric powder was weighed using an analytical balance (Kern ALJ 120-4, Balingen, Germany) and 500 mL of 70% ethanol was used as the solvent in the extraction. The extraction duration was set to 6 h at 70 °C. The extracted solution was then concentrated using a rotary evaporator (IKA RV 8 V-C, Asynt Ltd., Ely, UK) at 45 °C for 1 h. The concentrated product was freeze-dried, and the crude curcuminoid extract contained 5 g, corresponding to an extraction yield of 10% (*w/w*), which is consistent with previously reported yields for ethanol-based Soxhlet extractions of *Curcuma longa*.

To confirm the extraction process, Thin-Layer Chromatography (TLC) was performed as described previously [17]. A silica gel TLC plate was used for the stationary phase, while chloroform–dichloromethane–methanol (13:6:1) was used as the mobile phase. The observation was taken under UV (365 nm) light.

2.4. Characterization of Functional Groups of the Extract

2.4.1. FT-IR Analysis

The Fourier Transform Infrared (FT-IR) spectrum of the curcuminoid extract was recorded using an FTIR-ATR spectrophotometer (Horizon ABB-MB 3000 ATR, (ABB, Zurich, Switzerland)) equipped with a diamond ATR accessory. The spectra were measured in transmittance mode over 400–4000 cm^{−1}, with 64 scans per spectrum and a nominal resolution of 16 cm^{−1}. Data analysis was performed using Horizon MB software and OriginLab software (Origin 2022b, Build 9.9.5, OriginLab Corporation, Northampton, MA, USA).

2.4.2. LC–MS Analysis

Sample preparation for curcuminoid analysis

Dried curcuminoid extract (2 mg) was reconstituted in 1 mL of methanol (2 mg/mL), followed by dilution with an equal volume of Milli-Q water (50%, *v/v*) to improve solubility and chromatographic performance. The sample was sonicated for 10 min at room temperature to ensure complete dissolution and then centrifuged at 10,000 rpm for 10 min to remove insoluble particulates. The supernatant was filtered through a 0.22 µm PTFE syringe filter (Agilent Technologies, Santa Clara, CA, USA) and transferred into LC–MS autosampler vials for analysis.

Liquid chromatography–mass spectrometry (LC–MS) analysis of curcuminoids

Qualitative analysis of curcuminoids was performed using an Agilent 6230 Quadrupole Time-of-Flight (Q-TOF) (Agilent Technologies, Santa Clara, CA, USA) mass spectrometer equipped with an electrospray ionization (ESI) source. Chromatographic separation was achieved using an Agilent Poroshell 120 EC-C18 column (150 mm × 4.6 mm, 2.7 µm particle size) (Agilent Technologies, Santa Clara, CA, USA) maintained at 30 °C. The flow rate was set to 0.3 mL/min, and the injection volume was 5 µL. The mobile phase consisted of

solvent A (water containing 0.1% formic acid and 5 mM ammonium acetate) and solvent B (acetonitrile containing 0.1% formic acid and 5 mM ammonium acetate). A gradient elution program was applied as follows: 10% B from 0 to 2 min, linear increase from 10% to 90% B over 2 to 15 min, held at 90% B from 15 to 20 min, returned to 10% B from 20 to 22 min, and re-equilibrated at 10% B from 22 to 28 min. Mass spectrometric detection was performed using ESI in negative ion mode. The source parameters were set as follows: drying gas (nitrogen) flow rate of 10 L/min at 325 °C, nebulizer pressure of 40 psi, capillary voltage of 3500 V, and fragmentor voltage of 130 V. Data were acquired over a mass range of m/z 100–1000 using full-scan MS acquisition. LC–MS data were acquired and processed using Agilent MassHunter software (version B.07.00). Compound annotation was supported by comparison with spectral databases (METLIN, etc.), as well as reference to published literature on curcuminoid phytochemistry. LC-MS assignments were based solely on accurate mass and database matching, as authentic standards were not co-injected for LC-MS analysis.

2.5. Bioassay Using Dust Mites

2.5.1. Collection of Dust Mites and Culturing

Dust was collected from floors, furniture, and beds using a portable hand-held vacuum cleaner. Collected dust material was placed on a glass Petri dish in the same preparation medium (10 cm diameter) and observed under a dissecting microscope (Labomed CZM4 Binocular Zoom Microscope, Labo America Inc., Tarzana, CA, USA) at 10 × 40 for the presence of dust mites. The dust mites were cultured for 1 week using a 1:1 mixture of tuna fish powder and dried yeast. The cultures were kept undisturbed to allow for population growth [17].

2.5.2. Assessing the Toxicity Levels of Curcuminoid Extract for Dust Mites

Six concentrations (0.1, 1, 2.5, 5, 7.5, and 10 mg/mL) of turmeric powder and curcuminoid extracts were prepared using 70% ethanol as the solvent. Impregnated filter papers with each concentration for two samples were prepared by dipping filter papers in 3 mL of each concentration separately, followed by total evaporation of the solvent (30 min). Impregnated papers with different concentrations were placed in Petri dishes (60 mm diameter), and a batch of 10 mites was introduced to each Petri dish. They were exposed for 24 h, and the mortality rate was calculated. The minimum concentration of the extracts that provides the highest mortality rate was determined. Filter papers dipped in 3 mL of 70% ethanol and allowed to completely evaporate for 30 min were used as vehicle control; no mortality was observed in this group after 24 h. The experimental setup was prepared 3 times for each concentration separately for both turmeric powder and curcuminoid extracts.

Dust mites treated with the concentration that produced the highest mortality rate were observed under a high-power microscope (Leica DM500, Wetzlar, Germany) equipped with a mounted camera. Their behavior was continuously monitored and recorded for 24 h.

Mortality Assessment and Criteria:

Mortality was defined based on the complete and irreversible cessation of all observable movement under stereomicroscopic examination. A mite was considered dead when no movement of legs, gnathosoma, or body was detected for a continuous observation period of 60 s, even after gentle mechanical stimulation using a fine needle. Mites that showed minimal but detectable movement were classified as alive. All assessments were performed at predetermined time intervals over the 24 h monitoring period to ensure consistency across experimental and control groups. To avoid misclassification of transient

immobilization as mortality, mites were re-observed after 5 min before final confirmation of death.

2.6. *In Vitro* Assays on Immune and Skin Cells

2.6.1. Cell Cultures

The HaCaT human epidermal keratinocytes (ATCC) (CLS Cell Lines Service GmbH, Eppelheim, Germany; Lot No. 300493-4820) and RAW264.7 murine macrophage cells (European Collection of Authenticated Cell Cultures [ECACC], Salisbury, UK; Catalogue No. 91062702) were maintained in Dulbecco's Modified Eagle's Medium (DMEM/F12; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% (*v/v*) heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) and an antibiotic mixture containing 100 U/mL penicillin and 100 µg/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). The cells were incubated at 37 °C in a humidified 5% CO₂ atmosphere (MCO-170AICUV-PE incubator, PHCbi, Ora-gun, Gunma, Japan). Initially, the cells were seeded in 25 cm² flasks and later expanded into 75 cm² flasks upon reaching 80–90% confluency, with an initial seeding density of 2×10^4 cells/mL. The culture medium was replaced every two to three days.

2.6.2. Cell Viability Assay

HaCaT (human keratinocytes) and RAW264.7 (murine macrophages) were seeded in 96-well plates (Corning, NY, USA) at a density of 2×10^4 cells/mL and incubated overnight at 37 °C under 5% CO₂ to ensure adherence. Stock curcuminoid extract (40 µg/µL in DMSO) was serially diluted in 1% DMEM to generate 10 concentrations (0.4 to 0.0000008 µg/µL). Test solutions were added to the wells at a 1:100 (*v/v*) ratio (DMSO–media). Each concentration was tested in quadruplicate (*n* = 4), alongside a no-treatment (NT) control (1% DMSO in media). Negative (Milli-Q water) and positive (70% ethanol) controls were included. After 24 h of treatment, the wells were aspirated, washed with PBS, and incubated with 10 µL of alamarBlue reagent in 90 µL of fresh medium for 2 h. Fluorescence was quantified (FLUOstar OPTIMA microplate reader, BMG LABTECH, Ortenberg, Germany) at 540/590 nm (excitation/emission).

2.6.3. Cell Proliferation Assay

Cell proliferation was assessed in HaCaT and RAW264.7 cells using the alamarBlue assay as previously described¹⁹, with HaCaT cells plated at 2×10^4 cells/mL in 96-well plates (Corning, NY, USA) using DMEM/10% FBS and incubated for 24 h at 37 °C/5% CO₂. Curcuminoid extracts (40 µg/µL stock in DMSO) were diluted to 0.001 µg/µL in culture media (maintaining 1% DMSO) and applied to cells for 24, 48, and 72 h. At each time point, the medium was replaced with 10 µL alamarBlue in 90 µL fresh media, followed by 4 h incubation and fluorescence measurement (540/590 nm; FLUOstar OPTIMA). Controls included mitomycin-C (0.004 µg/µL; negative), LPS (100 ng/mL; positive; *E. coli* O55:B5, MilliporeSigma, Saint Louis, MO, USA), and untreated cells (1% DMSO).

2.6.4. Cell Migration Assay

The migratory potential of HaCaT cells was evaluated using a scratch wound assay following established methods. Twenty cells (1×10^5 cells/mL) were seeded in 48-well plates (Corning) and cultured at 37 °C/5% CO₂ until reaching 90% confluency. Uniform wounds were created using a 200 µL sterile pipette tip, followed by PBS (1X) washes to remove debris. Test treatments consisted of curcuminoid extracts (0.001 µg/µL final concentration) prepared by adding 5 µL stock solution (in DMSO) to 495 µL DMEM per well (final DMSO concentration: 1%). Following 24 and 48 h incubation at 37 °C, cells were processed via: (1) PBS (1X) washing, (2) fixation with 3.7% paraformaldehyde (2 min),

(3) methanol permeabilization (15 min), and (4) staining with 1% crystal violet (Sigma C0775, Saint Louis, MO, USA; 10 min in 2% ethanol). Migration was documented using phase-contrast microscopy (10X; ZEISS Axiovert 5 (Oberkochen, Germany) with motorized stage), with wound areas (mm²) quantified at 0, 24, and 48 h using ZEN software (version 3.14, Carl Zeiss, Oberkochen, Germany). ImageJ (version 1.53a, National Institutes of Health, Bethesda, MD, USA) analysis determined morphological parameters (area, perimeter, Feret diameter, circularity). Wound closure percentage was calculated as follows:

$$[(\text{AreaT}_0 - \text{AreaT}_t) / \text{AreaT}_0] \times 100 \quad (1)$$

where T₀ = initial wound area and T_t = area at time points.

2.6.5. Microscopy and Image Analysis for Toxicity

The cytotoxic effects of the test compounds on cellular morphology were evaluated in HaCaT and RAW264.7 cells using phase-contrast microscopy. Cells were seeded at 1×10^5 cells/well in a 96-well microplate and cultured for 24 h at 37 °C under 5% CO₂. Curcuminoid extracts (40 µg/µL stock in DMSO) were diluted to final concentrations of 0.4, 0.001, and 0.0000001 µg/µL in complete culture media, maintaining a constant 1% DMSO concentration across all treatments. Following 24 h of exposure, cellular morphology was examined using an inverted phase-contrast microscope (ZEISS Axiovert 5, 20× magnification) equipped with motorized stages for image acquisition.

Propidium iodide cytotoxicity assay:

Cell viability was assessed in HaCaT cells using propidium iodide (PI) staining, where membrane-impermeant PI selectively labels non-viable cells by intercalating with nucleic acids. Water-insoluble curcuminoid extracts (40 µg/µL DMSO stock) were serially diluted in complete media to generate test concentrations (0.4, 0.2, 0.1, 0.05, 0.03, 0.01, 0.006, 0.003, and 0.001 µg/µL), maintaining 1% DMSO across all treatments. Following 24 h of incubation, cells were stained with PI (15 min, RT) and immediately imaged by means of fluorescence microscopy (ZEISS Axiovert 5, 20×). Controls included: 70% ethanol (positive control), mitomycin-C (0.04 µg/µL; negative control), 1% DMSO in media (vehicle control, NT-I), and media with 1 µL Milli-Q water (media-only control, NT-II).

2.6.6. Anti-Inflammatory Activity

Nitric Oxide (NO) Inhibition Assay

The anti-inflammatory potential of curcuminoid extract was evaluated through NO suppression in LPS-stimulated RAW264.7 macrophages (1×10^5 cells/mL in 96-well plates). Following overnight incubation (37 °C, 5% CO₂), cells were treated with curcuminoid extracts (0.04, 0.001, 0.00001 µg/µL final concentrations in 1% DMSO) ± LPS (100 ng/mL) for 24 h. After removing the supernatant and PBS wash, NO production was quantified via the Griess reaction, measuring nitrite accumulation at 540 nm (FLUOstar OPTIMA).

Determination of Phagocytosis Activity

Phagocytic capacity was determined by means of neutral red uptake assay (modified from Premarathna et al., 2023 [18]). RAW264.7 cells (1×10^5 cells/mL) were treated for 24 h with curcuminoid extracts (0.4, 0.001, 0.0000001 µg/µL in 1% DMSO), LPS (100 ng/mL; positive control), or MMC (0.04 µg/mL; negative control). Following incubation, cells were exposed to neutral red (0.075% in PBS/DMEM, 1:1 v/v) for 2 h at 37 °C. After PBS washing, the intracellular dye was extracted with 1% acetic acid/50% ethanol (overnight at 25 °C) and quantified at 540 nm. Phagocytosis rate was calculated as follows:

$$\% \text{ Phagocytosis} = [(\text{OD}_{\text{treat}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{nt}} - \text{OD}_{\text{blank}})] \times 100 \quad (2)$$

where:

OD_{treat} = treated cells

OD_{nt} = untreated control (Milli-Q water)

$OD_{\beta\text{lank}}$ = media background

2.6.7. Gene Expression

HaCaT and RAW264.7 cells were seeded at a density of 1×10^5 cells/mL in 24-well plates (Corning) and cultured in DMEM supplemented with 10% FBS for 24 h at 37 °C under 5% CO₂ atmosphere. Cells were treated with water-insoluble curcuminoid extracts prepared by diluting 5 µL of stock solution (0.001 µg/µL final concentration) in 495 µL culture medium per well, maintaining a final DMSO concentration of 1%. Lipopolysaccharide (100 µg/mL) and mitomycin-C (0.004 µg/mL) served as positive and negative controls, respectively. Following treatment periods of 24, 48, and 72 h, cDNA was synthesized using the SuperScript™ IV CellsDirect™ cDNA Synthesis Kit with subsequent quantification by means of NanoDrop 2000 spectrophotometry (Thermo Fisher Scientific, Wilmington, DE, USA). Quantitative PCR was performed in 20 µL reaction volumes containing PowerTrack™ SYBR™ Green Master Mix (10 µL), cDNA template (5 µL), and 1.5 µM forward and reverse primers for target genes (TNF-α, TGF-β) and the reference gene (β-actin), completed to volume with DNase/RNase-free water. Thermal cycling conditions consisted of initial denaturation (95 °C, 5 min), followed by 40 cycles of denaturation (95 °C, 15 s), annealing (56.8 °C, 45 s), and extension (72 °C, 30 s), with final melting curve analysis (55–95 °C). Relative gene expression was determined using the comparative CT method ($2^{-\Delta\Delta CT}$) normalized to β-actin expression [19].

2.7. Statistical Analysis

All data were analyzed using GraphPad Prism 10.0.2 (San Diego, CA, USA). Results are presented as the mean ± standard error of the mean (SEM) from at least four independent experiments (n = 4). Statistical comparisons between treatment groups and the non-treated (NT) control were performed using one-way or two-way ANOVA, followed by Dunnett's multiple comparisons test. Statistical significance was defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. For gene expression analysis, relative mRNA expression was normalized to β-actin and calculated using the $2^{-\Delta\Delta CT}$ method. Statistical comparisons between the curcuminoid-treated group (0.001 µg/µL) and the untreated control (NT) were performed using an unpaired, two-tailed Student's *t*-test. A *p*-value < 0.05 was considered statistically significant; ns indicates not significant ($p > 0.05$).

3. Results

3.1. Characterization of the Functional Groups in the Curcuminoid Extract

3.1.1. Thin-Layer Chromatography (TLC) Test for the Extract

The determination of the curcuminoid fingerprint in the curcuminoid extract was carried out with the TLC method using curcumin (CRM), desmethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) standards. The alignment of R_f values between the extracted compounds and the standards indicates that the turmeric extract contains curcumin, demethoxycurcumin, and bisdemethoxycurcumin. And the alignment of R_f values with the literature provides even more clarity in determining the curcuminoids in the sample [17]. The recorded R_f values were: curcumin ($R_f = 0.66$), demethoxycurcumin ($R_f = 0.39$), and bisdemethoxycurcumin ($R_f = 0.19$), using chloroform–dichloromethane–methanol (13:6:1) as the mobile phase under UV light at 365 nm.

3.1.2. Functional Group Characterization of Curcuminoid Extract

The FTIR-ATR spectrum of curcuminoid extract (Figure 2) shows stretching vibrations at 1571 cm^{-1} attributed predominantly to the overlapping stretching vibrations of alkenes ($\text{C}=\text{C}$) and carbonyl ($\text{C}=\text{O}$) character. It shows stretching vibration at $3200\text{--}3500\text{ cm}^{-1}$ due to O-H groups, $\text{C}=\text{C}$ aromatic stretching vibration at 1434 cm^{-1} , and a band at 1500 cm^{-1} attributed to the mixed vibrations, including stretching carbonyl bond vibrations $\nu(\text{C}=\text{O})$, in-plane bending vibrations around aliphatic $\delta\text{ CC-C}$, $\delta\text{ CC=O}$, and in-plane bending vibrations around aromatic $\delta\text{ CC-H}$ of keto and enol configurations, and stretching vibrations around aromatic $\nu\text{ C-C}$ bonds of keto and enolic form of curcuminoids [20]. Plane deformation vibration (ar) C-H can be seen at 952 cm^{-1} ($1250\text{--}950\text{ cm}^{-1}$), while the (ar) C-C group, expected in the range $3080\text{--}3030\text{ cm}^{-1}$, overlaps with the broad peak around 3389 cm^{-1} . The band at 1040 cm^{-1} corresponds to (ar) C-O-(al)C symmetric stretching vibrations, usually observed in the range $1075\text{--}1020\text{ cm}^{-1}$ [21].

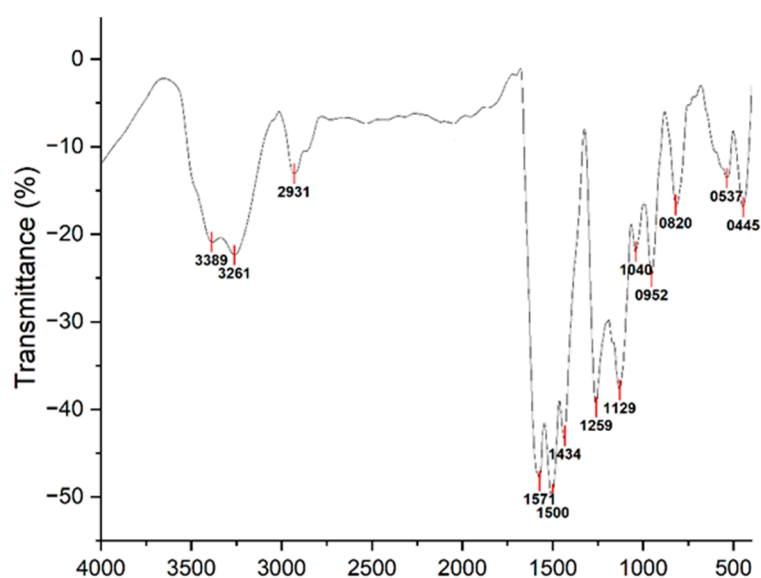


Figure 2. FTIR-ATR spectrum of freeze-dried powder of curcuminoid extract.

3.1.3. LC-MS Characterization of Curcuminoids

As shown in Figure 3, the three principal curcuminoids were successfully separated and identified using reversed-phase liquid chromatography–high-resolution mass spectrometry (LC-MS), following methodology similar to that reported by Núñez et al. 2020 [22]. The separation was achieved on a C18 column using a mobile phase gradient of water and acetonitrile containing 0.1% formic acid. Bisdemethoxycurcumin (BDMC), which lacks methoxy groups and is therefore the least hydrophobic (most polar) compound among the three, eluted first at a retention time of 15.926 min. Its deprotonated molecular ion ($[\text{M-H}]^-$) was detected at m/z 307.0973, corresponding to the exact molecular formula $\text{C}_{19}\text{H}_{15}\text{O}_4$ (calculated $[\text{M-H}]^-$: 307.0976). Demethoxycurcumin (DMC), containing one methoxy group and exhibiting intermediate hydrophobicity, eluted next at RT 16.126 min with an $[\text{M-H}]^-$ ion at m/z 337.1075. This mass corresponds to $\text{C}_{20}\text{H}_{17}\text{O}_5$ (calculated $[\text{M-H}]^-$: 337.1080), representing a mass increase of 30 Da compared to BDMC due to the addition of one methoxy group ($-\text{OCH}_3$). Curcumin, bearing two methoxy groups and therefore the most hydrophobic (least polar) compound among the three, eluted last at RT 16.359 min. Its $[\text{M-H}]^-$ ion was observed at m/z 367.1191, matching the exact mass for $\text{C}_{21}\text{H}_{19}\text{O}_6$ (calculated $[\text{M-H}]^-$: 367.1185). The successive mass differences of 30 Da between BDMC, DMC, and curcumin confirm the sequential addition of methoxy substituents. In reversed-phase chromatography, retention time increases with increasing hydrophobicity.

Therefore, the observed elution order (BDMC < DMC < curcumin) is consistent with the increasing number of methoxy groups, which enhance hydrophobicity. This interpretation aligns with published LC-MS analyses of curcuminoids [22,23].

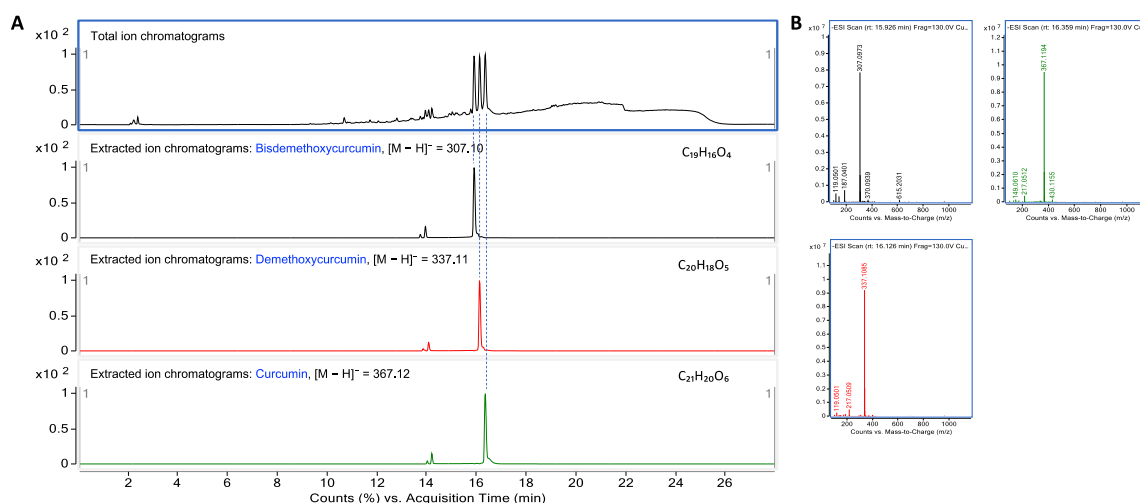


Figure 3. LC–MS characterization of the curcuminoid extract. (A) Total ion chromatogram (TIC) and extracted ion chromatograms (EICs) of bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC), and curcumin. (B) Negative-ion mass spectra of the corresponding chromatographic peaks, confirming the identities of the three curcuminoids.

3.2. Bioassay Using Dust Mites

Comparative Toxicity of Turmeric Powder and Curcuminoid Extract on House Dust Mites

The mortality rates of dust mites varied significantly depending on the type of extract and its concentration. Turmeric powder showed no acaricidal activity at any of the tested concentrations (0.1, 1, 2.5, 5, 7.5, and 10 mg/mL), with all replicates recording 0.0% mortality (mean mortality: $0.0\% \pm 0.0$). Similarly, curcuminoid extract at concentrations of 0.1 and 1 mg/mL showed no mortality (mean mortality: $0.0\% \pm 0.0$). At 2.5, 5.0, and 7.5 mg/mL, curcuminoid extract produced progressive mortality of 25.0%, 60.0%, and 85.0%, respectively, demonstrating a clear dose-dependent response. Exposure to curcuminoid extract at 10 mg/mL resulted in 100% mortality across all replicates (mean mortality: $100.0\% \pm 0.0$). These findings indicate that curcuminoid extract, but not crude turmeric powder, exerts a measurable acaricidal effect against house dust mites at higher concentrations, achieving complete mortality at 10 mg/mL within 24 h.

The selection of ten mites per condition was based on its use as a standardized experimental unit that allowed consistent replication and comparative analysis across treatments. This number ensured sufficient sensitivity to detect mortality differences while maintaining feasibility for continuous microscopic observation and accurate mortality scoring over a 24 h exposure period. The batch size also minimized handling stress and misclassification, thereby improving data reliability and reproducibility.

A two-way analysis of variance (ANOVA) revealed a statistically significant main effect of extract type ($F(1,24) = 162.00$; $p < 0.001$), a significant main effect of concentration ($F(5,24) = 174.75$, $p < 0.001$), and a significant interaction between extract type and concentration ($F(5,24) = 171.20$, $p < 0.001$). Post hoc comparisons using Tukey's HSD test indicated that the mortality rate for curcuminoid extract at 10 mg/mL was significantly higher ($p < 0.001$) than all other groups. To clarify the significant interaction effect, simple effects analysis was performed by conducting one-way ANOVA for each extract type separately at each of the six individual concentration levels (0.1, 1, 2.5, 5, 7.5, and 10 mg/mL), followed by Tukey's HSD pairwise comparisons between curcuminoid extract and turmeric

powder at each concentration. This approach confirmed that the two extract types differed significantly only at 10 mg/mL ($p < 0.001$), while no significant difference was detected at the five lower concentrations, where both extracts produced 0% mortality. These findings indicate that curcuminoid extract, but not turmeric powder, exerts a potent acaricidal effect at higher concentrations and may serve as a promising natural control agent against dust mites in household environments.

Figure 4 illustrates the mortality rates of dust mites following 24 h exposure to increasing concentrations (0.1, 1, 2.5, 5, 7.5, and 10 mg/mL) of curcuminoid extract and turmeric powder. A clear dose-dependent increase in mortality was observed for curcuminoids, reaching 100% at the highest concentration tested (10 mg/mL). In contrast, turmeric powder showed no acaricidal activity at any concentration, with 0% mortality consistently observed. Each point represents the mean of three replicates ($n = 3$). These results demonstrate the acaricidal effect of curcuminoid extract and suggest its potential application as a natural agent for controlling dust mites in indoor environments. The experimental observations, recorded with a microscope-equipped camera system, revealed a progressive decline in mite activity over time, culminating in mortality within 24 h. The behavioral analysis indicated a gradual reduction in their movement.

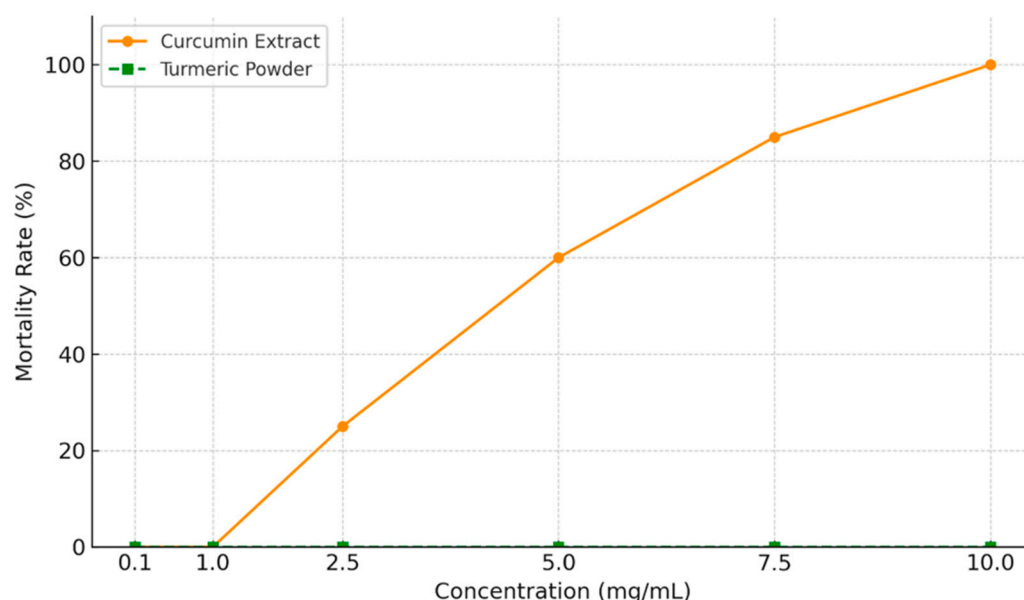


Figure 4. Comparative dose–response curves of curcuminoid extract and turmeric powder against house dust mites after 24 h exposure.

3.3. *In Vitro* Effects on Immune and Skin Cells

3.3.1. Effect of Curcuminoids on Cell Viability

Curcuminoids demonstrated a concentration-dependent cytotoxic effect in both HaCaT keratinocytes and RAW264.7 macrophages after 24 h of exposure, with clear thresholds for non-toxic concentrations identified in each cell type. In HaCaT cells, higher concentrations ($\geq 0.1 \mu\text{g}/\mu\text{L}$) significantly reduced viability, with values of $23.30 \pm 1.44\%$ at $0.4 \mu\text{g}/\mu\text{L}$ and $27.71 \pm 2.49\%$ at $0.2 \mu\text{g}/\mu\text{L}$ (NT-I, $100.0 \pm 0.00\%$; $p < 0.0001$). (Figure 5A). Cell viability gradually improved with decreasing concentrations, reaching $90.85 \pm 3.23\%$ at $0.001 \mu\text{g}/\mu\text{L}$, which was statistically indistinguishable from the control ($p = 0.0605$). Thus, $0.001 \mu\text{g}/\mu\text{L}$ was established as the highest non-cytotoxic concentration suitable for further skin-related assays, such as wound healing or anti-inflammatory studies. Similarly, RAW264.7 macrophages exhibited significant cytotoxicity at higher concentrations (0.4 – $0.013 \mu\text{g}/\mu\text{L}$), with viability ranging between 47.06 and 54.75% ($p < 0.0001$) (Figure 5A).

A shift toward non-toxic effects began at 0.003 $\mu\text{g}/\mu\text{L}$ ($83.43 \pm 7.35\%$; $p > 0.05$), and viability was fully restored at 0.001 $\mu\text{g}/\mu\text{L}$ ($94.63 \pm 3.50\%$) and lower. Therefore, 0.001 $\mu\text{g}/\mu\text{L}$ was identified as the highest safe concentration for RAW264.7 cells, balancing immunomodulatory potential with minimal cytotoxicity.

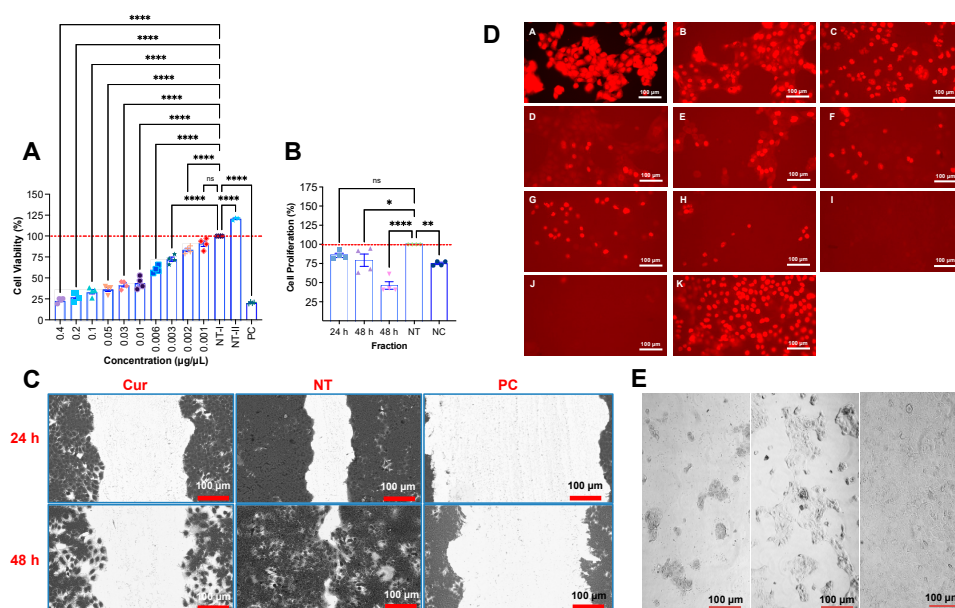


Figure 5. Biological effects of curcuminoid extracts (0.4–0.001 $\mu\text{g}/\mu\text{L}$) on HaCaT cells. (A) Cell viability of HaCaT cells treated with curcuminoid extracts. (B) Proliferation of RAW264.7 cells treated with 0.001 $\mu\text{g}/\mu\text{L}$ curcuminoid extract at 24 h, 48 h, and 72 h (alarmarBlue assay). (C) In vitro wound healing assay of HaCaT cells at 24 h: Curcuminoid-treated (0.001 $\mu\text{g}/\mu\text{L}$), untreated (NT), and (D) propidium iodide (PI) staining of HaCaT cells after 24 h curcuminoid exposure at different concentrations to evaluate cytotoxicity (D-A: 0.4 $\mu\text{g}/\mu\text{L}$, D-B: 0.2 $\mu\text{g}/\mu\text{L}$, D-C: 0.1 $\mu\text{g}/\mu\text{L}$, D-D: 0.05 $\mu\text{g}/\mu\text{L}$, D-E: 0.03 $\mu\text{g}/\mu\text{L}$, D-F: 0.01 $\mu\text{g}/\mu\text{L}$, D-G: 0.006 $\mu\text{g}/\mu\text{L}$, D-H: 0.003 $\mu\text{g}/\mu\text{L}$, D-I: 0.001 $\mu\text{g}/\mu\text{L}$, D-J: NT—No treatment, D-K: PC—Positive control (70% ethanol to kill cells)). (E) Microscopic observation (20X objective) of HaCaT cell morphology and proliferation at 24 h post-treatment. NT refers to no treatment (1% DMSO or Milli-Q water in culture media, as appropriate). A 70% ethanol solution was used as a positive control (PC) for cell death. Values are expressed as mean $\pm$ SE. Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparison test: **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$. Asterisks indicate significant differences, and “ns” indicates no significant difference from the respective control group.

These findings underscore the cell-type-specific sensitivity of curcuminoids and highlight the importance of precise dose selection for targeted biological effects. While higher concentrations may be reserved for cytotoxic or antiproliferative applications (anticancer strategies, etc.), sub-cytotoxic doses (≤ 0.001 – 0.003 $\mu\text{g}/\mu\text{L}$) show promise for therapeutic or cosmetic purposes where cell viability must be preserved. In particular, these lower concentrations could be harnessed in formulations designed to enhance macrophage function, such as phagocytic clearance of environmental allergens, including dust mite antigens, which are implicated in asthma and atopic dermatitis. Topical or aerosolizable products containing curcuminoids at these safe doses may offer novel anti-inflammatory or immune-supportive benefits in household, dermatological, or respiratory applications [24,25].

3.3.2. Impact on HaCaT Cell Proliferation and Migration

Treatment of HaCaT keratinocytes with curcuminoids at a concentration of 0.001 $\mu\text{g}/\mu\text{L}$ resulted in a time-dependent reduction in cell proliferation over a 72 h period (Figure 5B), as assessed using the alamarBlue assay. At 24 h, a slight, non-significant decrease in prolifer-

ation was observed ($85.66 \pm 2.82\%$) compared to the untreated control (NT; $100.0 \pm 0.00\%$, $p = 0.1473$). By 48 h, proliferation declined further to $79.27 \pm 8.09\%$, which was statistically significant ($p = 0.0206$). A marked and highly significant inhibition was observed at 72 h, with cell proliferation reduced to $46.19 \pm 5.00\%$ ($p < 0.0001$). Despite the decreased proliferation, there was no evidence of cytotoxicity at this concentration, suggesting a cytostatic rather than cytotoxic effect. These findings indicate that curcuminoids at $0.001 \mu\text{g}/\mu\text{L}$ are non-toxic to HaCaT cells but can modulate keratinocyte proliferation over time. Such a profile supports their potential application in dermatological and cosmetic formulations targeting hyperproliferative skin conditions or age-associated epidermal changes.

The ability to gently suppress keratinocyte proliferation without inducing cell death may also be advantageous in anti-ageing products, where modulation of epidermal turnover and support of barrier function are desired [24]. In addition, the results suggested that curcuminoids at $0.001 \mu\text{g}/\mu\text{L}$ exert a modest inhibitory effect on HaCaT cell migration (Figure 5C), potentially through cytostatic mechanisms. Such activity may be advantageous in contexts where controlled keratinocyte migration is desirable, such as in hyperproliferative or inflammatory skin conditions [26,27].

3.3.3. Cell Death Analysis by Means of Microscopic Observation and Propidium Iodide (PI) Staining

Cells treated with higher concentrations of curcuminoids, specifically $0.4 \mu\text{g}/\mu\text{L}$, $0.2 \mu\text{g}/\mu\text{L}$, and $0.1 \mu\text{g}/\mu\text{L}$ (Figure 5D-(A–C)), exhibited increased PI-positive staining, indicative of compromised membrane integrity and elevated cytotoxicity. Morphological signs of cell death, including cell shrinkage and membrane disruption, were visibly pronounced at these doses. In contrast, lower concentrations, such as $0.03 \mu\text{g}/\mu\text{L}$, $0.01 \mu\text{g}/\mu\text{L}$, $0.006 \mu\text{g}/\mu\text{L}$, $0.003 \mu\text{g}/\mu\text{L}$, and particularly $0.001 \mu\text{g}/\mu\text{L}$ (Figure 5D-(E–I)), showed minimal PI uptake, with cell morphology remaining largely intact and comparable to the no-treatment control (NT) (Figure 5D-(J)). These findings suggest that curcuminoids exert a dose-dependent cytotoxic effect on HaCaT cells, with concentrations of $\geq 0.1 \mu\text{g}/\mu\text{L}$ demonstrating clear toxicity. Therefore, the lowest tested concentration of $0.001 \mu\text{g}/\mu\text{L}$ appears non-toxic (Figure 5E) and is deemed suitable for further studies.

3.3.4. Anti-Inflammatory Effects of Curcuminoids Effects on Phagocytic Activity

Phagocytic activity of RAW264.7 macrophage cells following 24 h of treatment with curcuminoid extracts was evaluated across a wide concentration range (0.4 to $0.000001 \mu\text{g}/\mu\text{L}$). The results demonstrated a concentration-dependent effect on phagocytic function. Specifically, treatments with $0.001 \mu\text{g}/\mu\text{L}$ ($61.42 \pm 5.48\%$), $0.0004 \mu\text{g}/\mu\text{L}$ ($45.31 \pm 0.13\%$), and $0.0002 \mu\text{g}/\mu\text{L}$ ($69.31 \pm 0.98\%$) showed a statistically significant reduction or no substantial change in phagocytosis compared to the untreated control (NT-I: $100.0 \pm 0.00\%$) (Figure 5B), as confirmed by Dunnett's multiple comparisons test ($p < 0.01$ to $p < 0.0001$).

In contrast, higher concentrations such as $0.4 \mu\text{g}/\mu\text{L}$ ($8.39 \pm 2.00\%$), $0.2 \mu\text{g}/\mu\text{L}$ ($9.75 \pm 2.07\%$), and $0.1 \mu\text{g}/\mu\text{L}$ ($10.14 \pm 3.12\%$) significantly suppressed phagocytic activity ($p < 0.0001$), indicating potential cytotoxic or immunosuppressive effects at these levels. The optimal curcuminoid concentration range of 0.0002 – $0.001 \mu\text{g}/\mu\text{L}$ effectively enhances innate immune function without cytotoxicity, suggesting strong therapeutic potential. This immunomodulatory window could particularly benefit conditions involving macrophage dysfunction, such as dust mite allergies, where impaired phagocytosis contributes to chronic inflammation [2]. The combination of immune-enhancing effects and excellent safety profile at these concentrations supports potential applications in both environmental allergen mitigation and dermatological formulations for sensitive populations.

These findings position curcuminoids as promising natural agents for immune-related disorders requiring both efficacy and tissue compatibility.

Inhibition of Nitric Oxide Production

Nitric oxide (NO) production was assessed in LPS-stimulated RAW264.7 macrophages to evaluate the anti-inflammatory potential of curcuminoid extracts at varying concentrations: Cur-1 (0.01 $\mu\text{g}/\mu\text{L}$), Cur-2 (0.001 $\mu\text{g}/\mu\text{L}$), and Cur-3 (0.0001 $\mu\text{g}/\mu\text{L}$) (Figure 5C). Among the tested groups, Cur-2 showed the most significant suppression of NO levels ($2.50 \pm 0.38 \mu\text{M}$), with an adjusted p -value < 0.0001 compared to the LPS-treated control ($20.01 \pm 0.56 \mu\text{M}$), indicating strong anti-inflammatory activity at this low concentration. Cur-1 ($25.61 \pm 1.67 \mu\text{M}$) exhibited a moderate yet statistically significant reduction in NO production ($p = 0.0012$), while Cur-3 ($20.38 \pm 0.38 \mu\text{M}$) showed no significant difference compared to the LPS group, suggesting reduced efficacy at the lowest concentration. These findings indicate a dose-dependent inhibitory effect of curcuminoids on NO production, with 0.001 $\mu\text{g}/\mu\text{L}$ (Cur-2) identified as the most effective non-cytotoxic and anti-inflammatory concentration. Given the well-established role of NO in the pathogenesis of inflammatory conditions such as asthma and allergic responses, curcuminoids at this concentration could be explored as potential bioactive agents for respiratory health [28]. Specifically, they may offer benefits in controlling inflammation caused by asthma-inducing allergens, such as house dust mites, by mitigating macrophage-driven NO release. Furthermore, due to their low cytotoxicity and anti-inflammatory effects (in vivo), curcuminoid formulations at optimized doses may also hold promise in cosmeceutical applications, particularly in products designed to address skin inflammation, oxidative stress, and sensitive skin conditions [29,30].

3.3.5. Modulation of Gene Expression by Curcuminoids

The results demonstrate that the tested compounds exert immunomodulatory effects without compromising cell viability. As shown in Figure 6A, cell viability remained above 80% across all tested concentrations, with most treatments maintaining or even slightly enhancing viability compared to the untreated control, whereas the negative control showed a marked reduction in viability. Phagocytic activity (Figure 6B) increased in a dose-dependent manner, with several concentrations surpassing the baseline of untreated cells, indicating stimulation of macrophage functional activity. However, nitric oxide (NO) production (Figure 6C) was significantly lower in cells treated with curcuminoid extract 0.001 $\mu\text{g}/\mu\text{L}$ than in the LPS control group ($p < 0.0001$). In contrast, curcuminoid extract 0.01 $\mu\text{g}/\mu\text{L}$ treatment of RAW264.7 cells suggested an induction of pro-inflammatory signaling. The results for curcuminoid extract 0.001 $\mu\text{g}/\mu\text{L}$ were further supported by the decreased expression of TNF- α , indicating that this compound exhibits anti-inflammatory properties. TGF- β expression showed no significant change across the tested concentrations in RAW264.7 cells (Figure 6E). Microscopic examination of cell morphology (Figure 6D) further supported these findings, showing healthy, adherent, and well-spread cells under treatment, whereas the untreated and negatively impacted groups displayed reduced cell density and altered morphology. Collectively, these results suggest that the tested compounds (curcuminoids, etc.) help maintain macrophage functions such as modulating inflammatory mediators, reducing NO and TNF- α levels, and exhibiting overall anti-inflammatory potential.

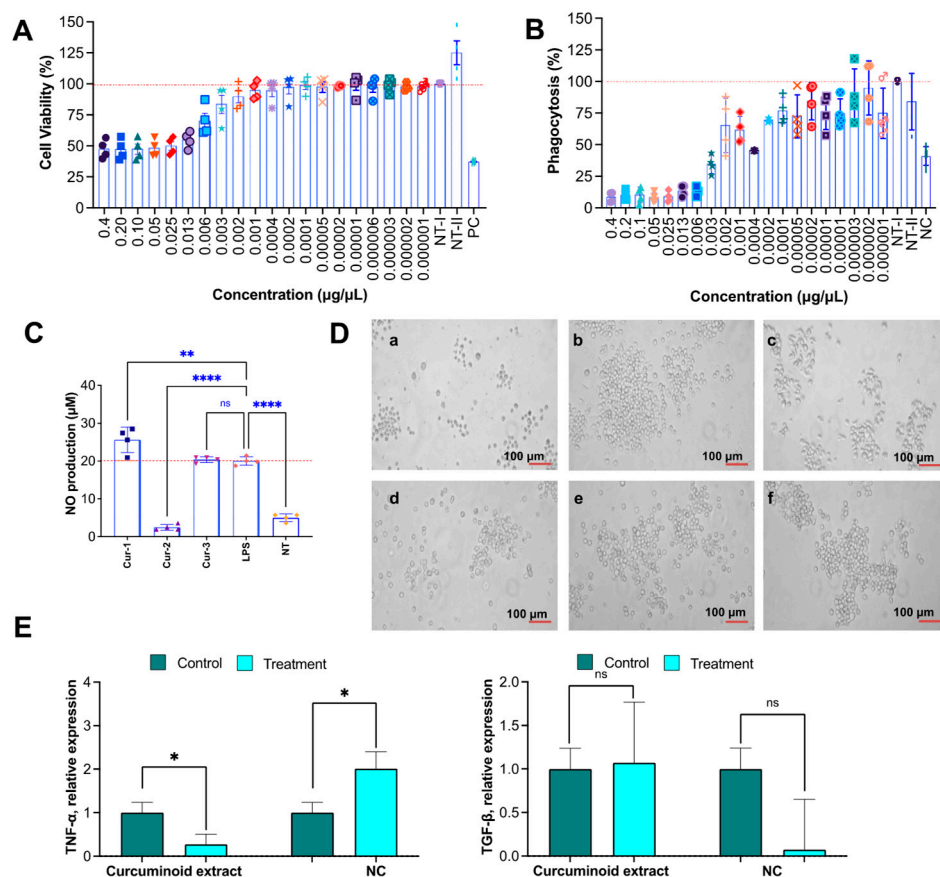


Figure 6. Effects of curcuminoid extracts (0.4–0.0000001 µg/µL) on RAW264.7 murine macrophage cells. (A) Cell viability assessed after treatment with curcuminoid extracts. (B) Phagocytosis activity of RAW264.7 cells treated with the same concentration range. NT-I: no treatment (1% DMSO in culture media); NT-II: no treatment (Milli-Q water); PC: 70% ethanol (positive control, where applicable); Mito–Mitomycin-C (0.004 µg/µL) is used as a negative control (NC). (C) Nitric oxide (NO) production in LPS-stimulated RAW264.7 cells treated with curcuminoid extracts at different concentrations: Cur-1 (0.01 µg/µL), Cur-2 (0.001 µg/µL), and Cur-3 (0.0001 µg/µL). LPS (100 ng/mL) was used for stimulation, and Milli-Q water was used as the untreated control. Data are expressed as mean ± SE. Statistical analysis was performed using one-way ANOVA followed by Dunnett’s multiple comparison test. **** $p < 0.0001$, ** $p < 0.01$. Asterisks indicate significant differences; (ns) indicates no significant difference from the respective control. (D) Microscopic observation (20X objective) of RAW264.7 cell morphology and proliferation at 24 h: a—0.4 µg/µL, b—NT-I (1% DMSO in media), c—NT-II (Milli-Q water in complete media), d—0.001 µg/µL, e—0.00001 µg/µL, f—0.0000001 µg/µL. (E) Relative gene expression of TNF-α and TGF-β in RAW264.7 cells treated with curcuminoid extract (0.001 µg/µL) for 24 h. Gene expression was normalized to β-actin and expressed relative to the untreated control (NT). Data are expressed as mean ± SE. Statistical analysis was performed using an unpaired two-tailed t -test. * $p < 0.05$ indicates significant difference from untreated control; (ns) indicates no significant difference.

4. Discussion

This study provides the first integrated evaluation of the dual functionality of curcuminoids extracted from rhizomes of *Curcuma longa*, combining acaricidal efficacy against house dust mites (HDMs) with anti-inflammatory effects in immune and epithelial cells. By addressing both the environmental source of allergens and the host inflammatory response, our findings highlight curcuminoids as promising candidates for eco-friendly allergy management strategies.

The curcuminoid extract demonstrated acaricidal activity, achieving 100% mortality of HDMs at 10 mg/mL, whereas turmeric powder exhibited no activity at equivalent

concentrations. This suggests that purified curcuminoids, rather than crude turmeric, are primarily responsible for the acaricidal effects. Similar bioactivity has been documented in other arthropods, where curcumin exerted insecticidal actions against *Aedes aegypti* mosquitoes [11], agricultural pests such as *Spodoptera litura*³⁴, and stored grain pests [12]. Exposure to curcuminoid extracts resulted in 100% mortality of house dust mites at a minimum effective concentration of 8 mg/mL, as determined through a concentration series.

When interpreting the acaricidal efficacy of the curcuminoid extract observed in this study in the context of previously reported natural and synthetic acaricides, several plant-derived essential oils, including clove, eucalyptus, and rosemary oil, have demonstrated high acaricidal activity against *Dermatophagoides* spp., often achieving complete mortality at considerably lower doses than that required for the curcuminoid extract in the present study [31]. Similarly, cinnamaldehyde, a major monoterpene constituent of cinnamon oil, has exhibited acaricidal potency exceeding that of the synthetic acaricide benzyl benzoate [32]. In contrast, ethanolic neem (*Azadirachta indica*) extract has shown comparatively modest acaricidal activity, requiring high concentrations (up to 50%) and extended exposure times of 24–96 h to achieve substantial mortality [33], an activity more comparable to that observed for the curcuminoid extract in the present study. Direct comparison across these studies is complicated by differences in bioassay format, solvent system, and concentration units (e.g., $\mu\text{g}/\text{cm}^2$ vs. mg/mL); nonetheless, these findings suggest that the curcuminoid extract exhibits acaricidal activity within the range reported for other moderately active plant-derived compounds, rather than the high potency observed for certain essential oils. This positions curcuminoids as a promising candidate for further optimization, for example, through encapsulation or formulation strategies to enhance bioavailability and acaricidal efficacy, rather than as a direct replacement for highly potent synthetic or essential oil-based acaricides at their current concentrations.

Microscopic observations before and after treatment confirmed the absence of repellent effects, indicating that the mites did not avoid the treated areas. This suggests that the curcuminoids exert their acaricidal effects through direct toxicity rather than deterrence. At lower concentrations, no mortality was observed, which may be attributed to the mites' inherent tolerance mechanisms. House dust mites normally survive for several weeks to months under optimal conditions, and no mortality was observed in untreated or vehicle control groups during the 24 h assay period. Therefore, the rapid mortality observed following curcuminoid exposure reflects a true toxic effect rather than age-related or spontaneous death. Similar to other arthropods, house dust mites possess adaptive strategies that enable them to survive under harsh environmental conditions. These include the ability to regulate water balance through specialized organs, such as the supracoxal glands, which concentrate electrolytes to absorb water vapor from the environment [34]. Additionally, insects have evolved various mechanisms to resist desiccation and thermal stress, including the production of heat-shock proteins and antifreeze proteins, which help maintain cellular integrity under extreme conditions [35]. However, when the concentration of the exposed chemical exceeds its maximum tolerance limit, rapid mortality could occur [36]. This phenomenon emphasizes the importance of determining the optimal concentration that balances efficacy and safety. According to published information on curcuminoid effects against *Tetranychus cinnabarinus* suggests that curcumin disrupts calcium homeostasis and detoxification pathways, leading to neuronal dysfunction and mortality [37]. These mechanisms may underlie the acaricidal effect observed here, though further mechanistic validation in HDMs is required. Importantly, acaricidal activity from natural sources, such as curcuminoids, offers a safer alternative to synthetic acaricides, which face limitations due to resistance and toxicity [12,38–40]. Therefore, further studies are warranted to elucidate the precise mechanisms underlying the acaricidal action of curcuminoids in the present

study and to evaluate their potential as eco-friendly alternatives to synthetic acaricides. Additionally, the acaricidal bioassay employed a single 24 h exposure endpoint. While mite behavior was continuously monitored over this period and a progressive, time-dependent decline in movement was observed culminating in full mortality at 10 mg/mL, formal time-gradient mortality quantification at multiple timepoints (e.g., 6 h, 12 h, 24 h, 48 h) was not performed. Such data would better characterize the onset and persistence of toxic effects and should be incorporated in future studies. Furthermore, a standard synthetic acaricide was not included as a positive control in the contact bioassay, and the anti-inflammatory assays were not compared against a reference agent such as dexamethasone or indomethacin. These omissions limit direct comparability with published studies and should be addressed in future work.

While the considerable acaricidal activity of curcuminoids was clearly demonstrated through controlled in vitro assays, we acknowledge that these results represent an initial proof-of-concept; consequently, household-level trials are essential to validate efficacy under real-world conditions—such as variable humidity and substrate complexity—while the development of advanced delivery systems, including nanoparticles or polymeric films, will be necessary to ensure long-term residual activity.

At non-toxic concentrations ($\leq 0.001 \mu\text{g}/\mu\text{L}$), curcuminoid extracts preserved the viability of HaCaT keratinocytes without significantly altering cell proliferation. In this study, treatment at $0.001 \mu\text{g}/\mu\text{L}$ resulted in reduced HaCaT cell migration, suggesting a cytostatic effect. This effect may be beneficial in hyperproliferative skin disorders (e.g., psoriasis and atopic dermatitis) but is not directly applicable to acute wound healing. However, previous studies have demonstrated that curcumin promotes re-epithelialization, reduces epidermal hyperplasia, and improves wound healing in inflammatory skin models [41,42].

In RAW264.7 macrophages, curcuminoids at $0.001 \mu\text{g}/\mu\text{L}$ did not enhance phagocytosis above untreated control levels (NT = 100%). Instead, phagocytic activity was reduced to 61–69% of NT levels, indicating that curcuminoids are less detrimental to macrophage phagocytic function than a known cytotoxic agent at the concentrations tested. Excessive NO release contributes to airway and skin inflammation in asthma and atopic dermatitis, whereas impaired phagocytosis reduces allergen clearance [2,43]. Our findings indicate that curcuminoids restore a balanced macrophage phenotype by supporting innate clearance functions while limiting pro-inflammatory mediator release. This dual activity aligns with previous studies showing that curcumin downregulates NF- κ B signaling, inducible nitric oxide synthase (iNOS), and cytokine production (TNF- α , IL-1 β), while promoting M2-like anti-inflammatory macrophage polarization [5].

Endotoxin contamination in the curcuminoid extract was not assessed; however, the sub-cytotoxic concentrations used ($\leq 0.001 \mu\text{g}/\mu\text{L}$) and the absence of unstimulated NO production suggest minimal endotoxin activity. Future studies should include endotoxin testing (e.g., LAL assay) for rigor. Several limitations of this study should be noted. The acaricidal bioassay did not include a standard synthetic acaricide as a positive control, and the anti-inflammatory assays were not compared against a reference agent such as dexamethasone or indomethacin. These omissions limit direct comparability with published studies and should be addressed in future work. By reducing mite burden and modulating downstream inflammatory responses, curcuminoids offer a dual-action strategy for mitigating allergic diseases, including asthma and atopic dermatitis. This is particularly relevant given that allergen exposure and immune dysregulation are tightly interlinked in allergic pathophysiology. Our results suggest potential applications in household sprays, textiles, or topical formulations designed to both lower HDM allergen levels and alleviate epithelial or macrophage-driven inflammation.

While the acaricidal activity was evident in vitro, household-level trials are needed to confirm efficacy under real environmental conditions. Similarly, the immunomodulatory effects observed in HaCaT and RAW264.7 cells should be validated in animal models of airway or skin allergy to confirm relevance in vivo. Another challenge is curcumin's low solubility and bioavailability; advanced delivery systems such as nanoparticles, micelles, or polymeric films have been shown to enhance curcumin stability and should be explored for optimized acaricidal and anti-inflammatory activity [44]. Simple formulation-based strategies, such as co-solvent systems (e.g., ethanol–glycerol or polyethylene glycol-based vehicles) or cyclodextrin-based inclusion complexes, may also offer practical, low-cost approaches to improving curcuminoid solubility for household applications. While the present study confirms the presence of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in the extract, the quantification of each individual compound was not performed. Future studies should include quantitative analysis of individual curcuminoids to establish structure–activity relationships and identify the specific compound(s) responsible for the observed acaricidal and anti-inflammatory activities. This will be essential for optimizing formulations and understanding the potential synergistic effects among curcuminoid constituents. Furthermore, accelerated stability testing assessing curcuminoid degradation under varying conditions of pH, temperature, and light exposure over time would help establish shelf-life and storage parameters relevant to real-world formulation development. Additionally, synergistic formulations with other natural acaricides (e.g., essential oils or piperine) may further improve efficacy. However, building on the in vitro findings of this study, future work should include simulated household environment trials to assess acaricidal efficacy under realistic conditions (e.g., variable humidity, surface types, and mite population density), as well as small-scale animal models of HDM-induced allergic airway or skin inflammation to validate the immunomodulatory effects observed in HaCaT and RAW264.7 cells. This establishment can be taken as a proof-of-concept for the dual acaricidal and anti-inflammatory bioactivity of curcuminoid extract, prior to undertaking resource- and time-intensive in vivo and field-based studies.

Our findings demonstrate that curcuminoid extracts exert measurable acaricidal effects against HDMs at higher concentrations, while at sub-cytotoxic levels, they modulate immune and skin cell functions relevant to inflammation and tissue repair.

5. Conclusions

In summary, our findings highlight *Curcuma longa* curcuminoid extracts as dual-action agents capable of reducing dust mite survival while modulating immune and skin cell responses relevant to inflammation. This dual bioactivity underscores their potential for development into natural household and therapeutic products aimed at allergen reduction and inflammation management. By bridging environmental control and cellular immunomodulation, curcuminoids represent a promising, eco-friendly approach to addressing the rising burden of allergic diseases.

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Abbreviations

HDM	House Dust Mites
CUR	Curcumin
DMC	Desmethoxycurcumin
BDMC	Bisdemethoxycurcumin

References

- Calderón, M.A.; Kleine-Tebbe, J.; Linneberg, A.; Blay, F.D.; de Rojas, D.H.F.; Virchow, J.C.; Demoly, P. House Dust Mite Respiratory Allergy: An Overview of Current Therapeutic Strategies. *J. Allergy Clin. Immunol. Pract.* **2015**, *3*, 843–855. [[CrossRef](#)] [[PubMed](#)]
- Jacquet, A. The Role of the House Dust Mite-Induced Innate Immunity in Development of Allergic Response. *Int. Arch. Allergy Immunol.* **2010**, *155*, 95–105. [[CrossRef](#)] [[PubMed](#)]
- Shishodia, S.; Sethi, G.; Aggarwal, B.B. Curcumin: Getting back to the roots. *Ann. N. Y. Acad. Sci.* **2005**, *1056*, 206–217. [[CrossRef](#)] [[PubMed](#)]
- Saifi, B.; Haftcheshmeh, S.M.; Feligioni, M.; Izadpanah, E.; Rahimi, K.; Hassanzadeh, K.; Mohammadi, A.; Sahebkar, A. An overview of the therapeutic effects of curcumin in reproductive disorders with a focus on the antiinflammatory and immunomodulatory activities. *Phytother. Res.* **2022**, *36*, 808–823. [[CrossRef](#)] [[PubMed](#)]
- Mohammadian Haftcheshmeh, S.; Khosrojerdi, A.; Aliabadi, A.; Lotfi, S.; Mohammadi, A.; Momtazi-Borojeni, A.A. Immunomodulatory Effects of Curcumin in Rheumatoid Arthritis: Evidence from Molecular Mechanisms to Clinical Outcomes. *Rev. Physiol. Biochem. Pharmacol.* **2021**, *179*, 1–29. [[CrossRef](#)] [[PubMed](#)]
- Haftcheshmeh, S.M.; Mirhafez, S.R.; Abedi, M.; Heydarlou, H.; Shakeri, A.; Mohammadi, A.; Sahebkar, A. Therapeutic potency of curcumin for allergic diseases: A focus on immunomodulatory actions. *Biomed. Pharmacother.* **2022**, *154*, 113646. [[CrossRef](#)] [[PubMed](#)]
- Kahkhaie, K.R.; Mirhosseini, A.; Aliabadi, A.; Mohammadi, A.; Mousavi, M.J.; Haftcheshmeh, S.M.; Sathyapalan, T.; Sahebkar, A. Curcumin: A modulator of inflammatory signaling pathways in the immune system. *Inflammopharmacology* **2019**, *27*, 885–900. [[CrossRef](#)] [[PubMed](#)]
- Momtazi-Borojeni, A.A.; Mosafer, J.; Nikfar, B.; Ekhlesi-Hundrieser, M.; Chaichian, S.; Mehdizadehkashi, A.; Vaezi, A. Correction to: Curcumin in Advancing Treatment for Gynecological Cancers with Developed Drug- and Radiotherapy-Associated Resistance. *Rev. Physiol. Biochem. Pharmacol.* **2019**, *176*, 131. [[CrossRef](#)] [[PubMed](#)]
- Tabaee, S.; Sahebkar, A.; Aghamohammadi, T.; Pakdel, M.; Dehabe, M.; Sobhani, R.; Alidadi, M.; Majeed, M.; Mirhafez, S.R. The Effects of Curcumin Plus Piperine Supplementation in Patients with Acute Myocardial Infarction: A Randomized, Double-Blind, and Placebo-Controlled Trial. In *Natural Products and Human Diseases: Pharmacology, Molecular Targets, and Therapeutic Benefits*; Sahebkar, A., Sathyapalan, T., Eds.; Springer International Publishing: Cham, Switzerland, 2021; pp. 199–211, ISBN 978-3-030-73234-9.
- Kausar, N.; Shier, W.T.; Ahmed, M.; Maryam; Albekairi, N.A.; Alshammari, A.; Saleem, M.; Imran, M.; Muddassar, M. Investigation of the insecticidal potential of curcumin derivatives that target the *Helicoverpa armigera* sterol carrier protein-2. *Heliyon* **2024**, *10*, e29695. [[CrossRef](#)] [[PubMed](#)]
- Matiadis, D.; Liggri, P.G.V.; Kritsi, E.; Tzioumaki, N.; Zoumpoulakis, P.; Papachristos, D.P.; Balatsos, G.; Sagnou, M.; Michaelakis, A. Curcumin Derivatives as Potential Mosquito Larvicidal Agents against Two Mosquito Vectors, *Culex pipiens* and *Aedes albopictus*. *Int. J. Mol. Sci.* **2021**, *22*, 8915. [[CrossRef](#)] [[PubMed](#)]
- Sureshbabu, A.; Smirnova, E.; Karthikeyan, A.; Moniruzzaman, M.; Kalaiselvi, S.; Nam, K.; Goff, G.L.; Min, T. The impact of curcumin on livestock and poultry animal's performance and management of insect pests. *Front. Vet. Sci.* **2023**, *10*, 1048067. [[CrossRef](#)] [[PubMed](#)]
- Seo, M.-D.; Kang, T.J.; Lee, C.H.; Lee, A.-Y.; Noh, M. HaCaT Keratinocytes and Primary Epidermal Keratinocytes Have Different Transcriptional Profiles of Cornified Envelope-Associated Genes to T Helper Cell Cytokines. *Biomol. Ther.* **2012**, *20*, 171–176. [[CrossRef](#)] [[PubMed](#)]
- Nakanishi-Matsui, M.; Yano, S.; Matsumoto, N.; Futai, M. Lipopolysaccharide induces multinuclear cell from RAW264.7 line with increased phagocytosis activity. *Biochem. Biophys. Res. Commun.* **2012**, *425*, 144–149. [[CrossRef](#)] [[PubMed](#)]
- Bisht, K.; Choi, W.H.; Park, S.Y.; Chung, M.K.; Koh, W.S. Curcumin enhances non-inflammatory phagocytic activity of RAW264.7 cells. *Biochem. Biophys. Res. Commun.* **2009**, *379*, 632–636. [[CrossRef](#)] [[PubMed](#)]

16. Vidanapathirana, N.P.; Subasinghe, S.; Sunil, K.; Ketippearachchi, K.G.; Siriwardana, A.J.M.C.M.; Bandusekara, B.S. Growth and yield performances of turmeric (*Curcuma longa*) grown in dry zone of Sri Lanka as affected by planting space, growing media and shade. *J. Agro-Technol. Rural Sci.* **2021**, *1*, 27–31. [CrossRef]
17. Syarifah, A.L.; Retnowati, R.; Sulistyarti, H. Characterization of the Curcuminoids Fingerprint Profile in Curcuma and Zingiber Genera by TLC–Digital Image Analysis. *J. Pure Appl. Chem. Res.* **2019**, *8*, 147–161. [CrossRef]
18. Premarathna, A.D.; Tuvikene, R.; Somasiri, M.; De Silva, M.; Adhikari, R.; Ranaheewa, T.H.; Wijesundara, R.; Wijesekera, S.K.; Dissanayake, I.; Wangchuk, P.; et al. A novel therapeutic effect of mannitol-rich extract from the brown seaweed *Sargassum ilicifolium* using in vitro and in vivo models. *BMC Complement Med. Ther.* **2023**, *23*, 26. [CrossRef] [PubMed]
19. Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
20. Ismail, E.; Younis, H.; Khalil, M. Synthesis and Characterization of some Ternary Metal Complexes of Curcumin with 1,10-phenanthroline and their Anticancer Applications. *J. Sci. Res.* **2014**, *6*, 509–519. [CrossRef]
21. Cocean, A.; Cocean, I.; Cimpoesu, N.; Cocean, G.; Cimpoesu, R.; Postolachi, C.; Popescu, V.; Gurlui, S. Laser Induced Method to Produce Curcuminoid-Silanol Thin Films for Transdermal Patches Using Irradiation of Turmeric Target. *Appl. Sci.* **2021**, *11*, 4030. [CrossRef]
22. Núñez, N.; Vidal-Casanella, O.; Sentellas, S.; Saurina, J.; Núñez, O. Characterization, Classification and Authentication of Turmeric and Curry Samples by Targeted LC-HRMS Polyphenolic and Curcuminoid Profiling and Chemometrics. *Molecules* **2020**, *25*, 2942. [CrossRef] [PubMed]
23. Kawano, S.; Inohana, Y.; Hashi, Y.; Lin, J.-M. Analysis of keto-enol tautomers of curcumin by liquid chromatography/mass spectrometry. *Chin. Chem. Lett.* **2013**, *24*, 685–687. [CrossRef]
24. Mo, Z.; Yuan, J.; Guan, X.; Peng, J. Advancements in Dermatological Applications of Curcumin: Clinical Efficacy and Mechanistic Insights in the Management of Skin Disorders. *Clin. Cosmet. Investig. Dermatol.* **2024**, *17*, 1083–1092. [CrossRef] [PubMed]
25. Sandersen, C.; Bienzle, D.; Cerri, S.; Franck, T.; Derochette, S.; Neven, P.; Mouytis-Mickalad, A.; Serteyn, D. Effect of inhaled hydrosoluble curcumin on inflammatory markers in broncho-alveolar lavage fluid of horses with LPS-induced lung neutrophilia. *Multidiscip. Respir. Med.* **2015**, *10*, 16. [CrossRef] [PubMed]
26. Dujic, J.; Kippenberger, S.; Hoffmann, S.; Ramirez-Bosca, A.; Miquel, J.; Diaz-Alperi, J.; Bereiter-Hahn, J.; Kaufmann, R.; Bernd, A. Low Concentrations of Curcumin Induce Growth Arrest and Apoptosis in Skin Keratinocytes Only in Combination with UVA or Visible Light. *J. Investig. Dermatol.* **2007**, *127*, 1992–2000. [CrossRef] [PubMed]
27. Sun, J.; Han, J.; Zhao, Y.; Zhu, Q.; Hu, J. Curcumin induces apoptosis in tumor necrosis factor- α -treated HaCaT cells. *Int. Immunopharmacol.* **2012**, *13*, 170–174. [CrossRef] [PubMed]
28. Pulido-Moran, M.; Moreno-Fernandez, J.; Ramirez-Tortosa, C.; Ramirez-Tortosa, M. Curcumin and Health. *Molecules* **2016**, *21*, 264. [CrossRef] [PubMed]
29. Frei, G.; Haimhoffer, Á.; Csapó, E.; Bodnár, K.; Vasvári, G.; Nemes, D.; Lekli, I.; Gyöngyösi, A.; Bácskay, I.; Fehér, P.; et al. In Vitro and In Vivo Efficacy of Topical Dosage Forms Containing Self-Nanoemulsifying Drug Delivery System Loaded with Curcumin. *Pharmaceutics* **2023**, *15*, 2054. [CrossRef] [PubMed]
30. Kasprzak-Drozd, K.; Niziński, P.; Hawrył, A.; Gancarz, M.; Hawrył, D.; Oliwa, W.; Pałka, M.; Markowska, J.; Oniszczyk, A. Potential of Curcumin in the Management of Skin Diseases. *Int. J. Mol. Sci.* **2024**, *25*, 3617. [CrossRef] [PubMed]
31. Saad, E.-Z.; Hussien, R.; Saher, F.; Ahmed, Z. Acaricidal activities of some essential oils and their monoterpenoidal constituents against house dust mite, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *J. Zhejiang Univ. Sci. B* **2006**, *7*, 957–962. [CrossRef] [PubMed]
32. Wang, Z.; Kim, H.-K.; Tao, W.; Wang, M.; Ahn, Y.-J. Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *J. Med. Entomol.* **2011**, *48*, 366–371. [CrossRef] [PubMed]
33. Hanifah, A.L.; Awang, S.H.; Ming, H.T.; Abidin, S.Z.; Omar, M.H. Acaricidal activity of *Cymbopogon citratus* and *Azadirachta indica* against house dust mites. *Asian Pac. J. Trop. Biomed.* **2011**, *1*, 365–369. [CrossRef] [PubMed]
34. Aggarwal, P.; Senthikumar, S. Dust Mite Allergy. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2025. Available online: <http://www.ncbi.nlm.nih.gov/books/NBK560718/> (accessed on 26 March 2025).
35. King, A.M.; MacRae, T.H. Insect Heat Shock Proteins During Stress and Diapause. *Annu. Rev. Entomol.* **2015**, *60*, 59–75. [CrossRef] [PubMed]
36. Leong, X.-Y.; Singham, G.V.; Shu-Chien, A.C.; Doggett, S.L.; Lee, C.-Y.; Leong, X.-Y.; Singham, G.V.; Shu-Chien, A.C.; Doggett, S.L.; Lee, C.-Y. Influences of Exposure Time and Mortality Assessment Interval on Bioassay Results of Insecticide-Resistant Tropical Bed Bugs (Hemiptera: Cimicidae). *Insects* **2020**, *11*, 640. [CrossRef] [PubMed]
37. Liu, X.; Wu, D.; Zhang, Y.; Zhou, H.; Lai, T.; Ding, W. RNA-Seq Analysis Reveals Candidate Targets for Curcumin against *Tetranychus cinnabarinus*. *BioMed Res. Int.* **2016**, *2016*, 2796260. [CrossRef] [PubMed]

38. Damodharan, S.; Poyilil, A.; Bimisha, C.M. Curcuma longa essential oil as an acaricidal agent for the control of cattle ectoparasitic tick-Rhipicephalus (Boophilus) annulatus. *Sci. Rep.* **2024**, *14*, 28138. [[CrossRef](#)] [[PubMed](#)]
39. Quadros, D.G.; Johnson, T.L.; Whitney, T.R.; Oliver, J.D.; Chávez, A.S.O.; Quadros, D.G.; Johnson, T.L.; Whitney, T.R.; Oliver, J.D.; Chávez, A.S.O. Plant-Derived Natural Compounds for Tick Pest Control in Livestock and Wildlife: Pragmatism or Utopia? *Insects* **2020**, *11*, 490. [[CrossRef](#)] [[PubMed](#)]
40. Bisht, N.; Fular, A.; Ghosh, S.; Nanyiti, S. Systematic review and meta-analysis of acaricidal properties of plant derived products against Ixodid ticks population. *Discov. Appl. Sci.* **2025**, *7*, 189. [[CrossRef](#)]
41. Chen, Q.; Tang, Y.; Deng, H.; Liang, B.; Li, H.; Li, Z.; Zhu, H.; Chen, L. Curcumin Improves Keratinocyte Proliferation, Inflammation, and Oxidative Stress through Mediating the SPAG5/FOXO1 Axis in an In Vitro Model of Actinic Dermatitis by Ultraviolet. *Dis. Markers* **2022**, *2022*, 5085183. [[CrossRef](#)] [[PubMed](#)]
42. Lee, H.-J.; Jeong, M.; Na, Y.-G.; Kim, S.-J.; Lee, H.-K.; Cho, C.-W.; Lee, H.-J.; Jeong, M.; Na, Y.-G.; Kim, S.-J.; et al. An EGF- and Curcumin-Co-Encapsulated Nanostructured Lipid Carrier Accelerates Chronic-Wound Healing in Diabetic Rats. *Molecules* **2020**, *25*, 4610. [[CrossRef](#)] [[PubMed](#)]
43. Wang, J.-Y. The Innate Immune Response in House Dust Mite-Induced Allergic Inflammation. *Allergy Asthma Immunol. Res.* **2013**, *5*, 68–74. [[CrossRef](#)] [[PubMed](#)]
44. Moballegh Nasery, M.; Abadi, B.; Poormoghdam, D.; Zarrabi, A.; Keyhanvar, P.; Khanbabaie, H.; Ashrafizadeh, M.; Mohammadinejad, R.; Tavakol, S.; Sethi, G. Curcumin Delivery Mediated by Bio-Based Nanoparticles: A Review. *Molecules* **2020**, *25*, 689. [[CrossRef](#)] [[PubMed](#)]

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