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EFFECTS OF FLAVONOIDS ON MARKERS OF TGF- β_1 -INDUCED METABOLIC, LYSOSOMAL, AND EPITHELIAL-MESENCHYMAL REMODELING IN EPITHELIAL CELLS

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Background. One of the major complications of COVID-19 is pulmonary fibrogenesis with an incompletely understood etiology, which is initiated by the activation of epithelial-mesenchymal transition (EMT) in the alveolar epithelium. Given that the leading principle of medicine remains “*primum non nocere*” (“first, do no harm”), the search for substances with a broad therapeutic potential combined with an exceptional safety profile is highly relevant. Such compounds include secondary plant metabolites, among which multifunctional citrus flavonoids have recently attracted considerable interest.



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The aim of this study was to investigate whether flavonoids can modulate TGF- β_1 -induced metabolic, lysosomal, and epithelial-mesenchymal remodeling in epithelial cells by assessing relevant molecular markers, as well as their antimicrobial activity in an experimental EMT model.

Materials and Methods. The inhibitory effects of hesperidin and hesperetin on EMT were investigated *in vitro* using the human type II alveolar epithelial cell line A549. EMT was induced by TGF- β_1 at concentrations of 2.0 and 10 ng/mL. Morphological changes in the cells were assessed by light microscopy. The expression of mesenchymal markers in cell lysates and supernatants was determined by Western blot analysis. Total protein content was measured using the Bradford assay. The antioxidant activity of the flavonoids was evaluated using the DPPH assay, whereas β -glucosidase activity (β -GA) in cell lysates was determined spectrophotometrically based on the hydrolysis of pNPG. The antimicrobial and antifungal activity of the flavonoids was assessed by determining their minimum inhibitory concentrations (MICs) against *Candida albicans* ATCC 90028, *Escherichia coli* ATCC 25922, and *Streptococcus agalactiae* ATCC 13813 using the broth microdilution method. Microbial growth was evaluated by changes in the turbidity of the medium after incubation in the appropriate culture media.

Results. Hesperidin caused a more pronounced reduction in the level of phosphorylated SMAD3 (p-SMAD3), an early molecular marker of EMT ($P^{**} < 0.05$). Both flavonoids, particularly hesperetin, significantly ($P^* < 0.05$) reduced the expression of mesenchymal proteins (collagen I and α -SMA), thereby inhibiting the myofibroblastic differentiation of epithelial cells. In addition, the aglycone, owing to its higher antioxidant activity, more markedly ($P^{**} < 0.05$) affected the expression of PAI-1 – a marker associated with oxidative stress and EMT. The flavonoids modulated the enhanced TGF β_1 -induced lysosomal metabolism of transformed cells, significantly ($P^* < 0.05$) reducing the lysosomal remodeling marker – β -GA. Hesperidin and hesperetin exhibited low to moderate antimicrobial and antifungal activity, which may be considered one of the factors potentially contributing to the maintenance of microbial homeostasis and the functional integrity of the epithelial barrier.

Conclusions. The obtained results indicate the ability of the citrus flavonoids hesperidin and hesperetin to suppress the expression of early signaling molecules and late markers in an experimental model of EMT. Hesperidin and its aglycone hesperetin modulate the TGF- β /SMAD3 signaling pathway and inhibit the metabolic and lysosomal remodeling associated with EMT, demonstrating their antimicrobial potential.

Keywords: hesperidin, hesperetin, SMAD3 signaling pathway, lysosomal-metabolic remodeling, antimicrobial and antifungal potential

LIST OF ABBREVIATIONS

EMT	– Epithelial-Mesenchymal Transition
MET	– Mesenchymal-Epithelial Transition
COVID-19	– Coronavirus Disease 2019
A549	– human lung adenocarcinoma cell line
TGF- β_1	– Transforming Growth Factor Beta 1
Hsd	– Hesperidin
Hst	– Hesperetin
SMAD3	– Mothers Against Decapentaplegic Homolog 3
p-SMAD3	– Phosphorylated SMAD3

α -SMA	– Alpha-Smooth Muscle Actin
ECM	– extracellular matrix proteins
PAI-1	– Plasminogen Activator Inhibitor-1
MIC	– Minimum Inhibitory Concentration
MHB	– Mueller–Hinton Broth
CAMHB	– Cation-Adjusted Mueller–Hinton Broth
CAMHB-LHB	– Cation-Adjusted Mueller–Hinton Broth with Lysed Horse Blood
LHB	– Lysed Horse Blood
CLSI	– Clinical and Laboratory Standards Institute
CFU	– Colony-Forming Unit(s)
MOPS	– 3-(N-Morpholino)propanesulfonic Acid
RPMI	– Roswell Park Memorial Institute medium
FBS	– Fetal Bovine Serum
SDS	– Sodium Dodecyl Sulfate
EDTA	– Ethylenediaminetetraacetic Acid
PVDF	– Polyvinylidene Fluoride
TBS-T/TBST	– Tris-Buffered Saline with Tween-20
AcrAB-TolC	– multidrug efflux pump
DPPH	– 2,2-Diphenyl-1-picrylhydrazyl
IC ₅₀	– Half Maximal Inhibitory Concentration
β -GA	– β -glucosidase activity
pNPG	– para-Nitrophenyl- β -D-glucopyranoside
pNP	– para-Nitrophenol
TFEB	– Transcription Factor EB (E-box binding protein)
LPS	– Lipopolysaccharide
Snail	– Snail Family Transcriptional Repressor
Slug	– Snail Family Transcriptional Repressor
Twist	– Twist Family BHLH Transcription Factor
ERK	– Extracellular Signal-Regulated Kinase
p38 MAPK	– p38 Mitogen-Activated Protein Kinase
JNK	– c-Jun N-terminal Kinase
PI3K/Akt	– Phosphoinositide 3-Kinase / Protein Kinase B (Akt)
NF- κ B	– Nuclear Factor kappa-light-chain-enhancer of activated B cells
Wnt/ β -catenin	– Wingless-related integration site / β -catenin signaling pathway
PLOD2	– Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 2
LOXL2	– Lysyl Oxidase Like 2
ZEB	– Zinc Finger E-Box Binding Homeobox
SD	– Standard Deviation

INTRODUCTION

An undesirable consequence in 44.9 % of individuals who have recovered from coronavirus disease COVID-19 is the development of pulmonary complications of incompletely understood etiology, which may progress to fibrosis and have limited therapeutic options (Hama Amin *et al.*, 2022). Epithelial-mesenchymal transition, as an initial stage of fibrosis, is primarily characterized by the deposition of excessive amounts of collagen and extracellular matrix components (elastic fibers, proteoglycans, fibronectin, and

fibrillar collagens) in the alveolar walls (Lang *et al.*, 2023), their remodeling (Gaikwad *et al.*, 2023), and the formation of myofibroblasts (Ji *et al.*, 2023).

This process was first described by Elizabeth Hay in 1968 as the loss of key characteristics of epithelial cells. EMT in lung tissue that develops after severe acute coronavirus infection during the early stages (from weeks to the first months after the acute phase) is partially or completely reversible (Pattarayan *et al.*, 2022; Gu *et al.*, 2023). The lung tissue microenvironment may recover if inflammation is halted and type II pneumocytes return to the epithelial phenotype. However, if the infection persists and the fibroblast-like state is stabilized through collagen deposition, post-COVID interstitial fibrosis develops, which is irreversible or only partially reversible (Hama Amin *et al.*, 2022; Gu *et al.*, 2023).

Currently approved antifibrotic drugs (pirfenidone and nintedanib) (Knüppel *et al.*, 2017; Hu *et al.*, 2023), unfortunately, cause numerous adverse effects (Zhang *et al.*, 2022), prompting ongoing research aimed at identifying and developing compounds with therapeutic properties. The discovery of compounds as potential modulators of EMT and enhancers of mesenchymal-epithelial transition (MET) opens new possibilities for the treatment of these pathogenic conditions (Lee *et al.*, 2021; Pryimak *et al.*, 2021; Song *et al.*, 2022; Ji *et al.*, 2023).

Given that the leading principle of medicine remains *primum non nocere* ("first, do no harm"), the search for compounds possessing a broad spectrum of therapeutic potential together with an exceptional safety profile (Haggag *et al.*, 2020; Choi *et al.*, 2022) continues to attract considerable attention. Such compounds include secondary plant metabolites, for which increasing evidence has emerged in recent years regarding their antifibrotic and other therapeutic effects (Bahri *et al.*, 2017; Pattarayan *et al.*, 2017; Lee *et al.*, 2021; Hasan *et al.*, 2022). Among these substances are the flavonoid hesperidin and its derivatives (Lin *et al.*, 2005; Hosawi *et al.*, 2023; Nesehi *et al.*, 2023).

To test the working hypothesis concerning the ability of flavonoids to inhibit EMT *in vitro* associated with post-COVID complications, hesperidin and its aglycone, hesperetin, were selected as the subjects of our study.

MATERIALS AND METHODS

Hesperidin (Hsd, 3',5,7-trihydroxy-4'-methoxyflavanone 7-rutinoside) (Cat. No. H5254) and hesperetin (Hst, 3',5,7-trihydroxy-4'-methoxyflavanone) (Cat. No. W 431300) were purchased from Sigma-Aldrich Co. (Switzerland). Both compounds were used without further purification, dissolved in a DMSO:H₂O solution at a 1:1 ratio. A dilution series of 0.5...75 µg/mL was prepared. The EMT process was induced with the cytokine Transforming Growth Factor Beta 1 (TGF-β₁) (Cat. No. 10804-HNAC, Sino Biological Inc., Beijing, China). The anionic dye Coomassie Brilliant Blue G-250 (Cat. No. 11475951) was obtained from Thermo Scientific, and the chromogenic substrate p-Nitrophenyl-β-D-glucopyranoside (Cat. No. 487507), Merck Life Science spol. s r.o.

Primary cell isolation and cell culture. Cell culture. To study the EMT process, the human lung adenocarcinoma alveolar basal epithelial cell line A549 was used as a model of alveolar type II lung epithelial cells. Cells were incubated in 12-well plates in RPMI 1640 medium (BioSera, France) supplemented with 10 % FBS, 1 % penicillin-streptomycin solution, and 1 % glutamine (Life Technologies, USA) at 37 °C in a humidified 5 % CO₂ incubator. A suspension of A549 cells in RPMI medium with 10 % FBS was

placed into each well of a 12-well plate at a density of at least $1 \cdot 10^6$ cells/well for 24 h. After that, the initial medium was replaced with RPMI, and the cells were subjected to serum starvation (1 % penicillin-streptomycin and 1 % glutamine, without FBS) supplemented with 0.5 % HEPES (Sigma-Aldrich, USA, No. H0887). The cells were exposed to TGF- β_1 (2.0...10.0 ng/mL) alone or in combination with Hsd or Hst (0.5...75 μ g/mL) for 2, 4, 8, and 72 h to assess their EMT-inhibitory capacity.

Western blot analysis. Protein from A549 cells was extracted and subjected to SDS–polyacrylamide gel electrophoresis for Western blot analysis. The levels of the proteins of interest were analyzed separately in cell lysates and culture supernatants according to their intracellular or secreted localization. The proteins pSMAD3/SMAD3, collagen, and α -SMA were assessed in cell lysates to determine their intracellular levels, whereas the concentration of the secreted protein PAI-1 was measured in culture supernatants.

Cell cultures were lysed on ice using a 1 % lysis buffer. The culture supernatant was collected and immediately centrifuged for 45 s at 10,000 rpm. After centrifugation, the supernatant was collected and mixed with Simple Buffer at a 1:3 ratio. In place of the removed supernatants, lysis buffer was added to the cells on ice; after several minutes, it was collected and centrifuged for 45 s at 10,000 rpm. The resulting supernatant was then collected and mixed with Simple Buffer at the same ratio as described for the initial supernatants. The lysis buffer was prepared using 9 mL EDTA, 1 mL Triton X-100 (10 %), and 5 μ L Protease Inhibitor Cocktail III. For the detection of phosphorylated SMAD3 (Mothers Against Decapentaplegic Homolog 3), a standard lysis buffer supplemented with a phosphatase inhibitor mixture was used, containing sodium orthovanadate (2 mM) and sodium fluoride (2 mM).

Before transferring the samples onto a polyvinylidene fluoride membrane (PVDF) (Cytiva Life Sciences™ Amersham, USA, 21204908 AB), the samples were heated at 95 °C for 5 min. Approximately 40 μ g of protein was loaded into each lane of the SDS-PAGE acrylamide gels. Depending on the proteins under investigation, the samples were separated on 8 %, 10 %, and 12 % gels and transferred onto a PVDF membrane. The membranes were blocked in 5 % dry milk with 0.1 % azide for 40 minutes, followed by three washes in Tris-buffered saline (TBS; pH 7.4). Incubation with primary antibodies was carried out for at least 2 h at room temperature using mono- and polyclonal antibodies. Specifically, rabbit polyclonal anti-Collagen I (Abcam, GB, Cat. No. ab292), rabbit polyclonal anti- α -SMA (alpha-smooth muscle actin) (Abcam, GB, Cat. No. ab5694), rabbit polyclonal anti-SMAD3 (Invitrogen, USA and Canada, Cat. No. PA5-17378), rabbit polyclonal anti-phospho-SMAD3 (Invitrogen, USA and Canada, Cat. No. PA5-12694), and rabbit polyclonal anti-PAI-1 (plasminogen activator inhibitor-1) (Abcam, GB, Cat. No. 66705) were used. After incubation with primary antibodies, the membranes were washed three times in Tris-buffered saline with Tween (TBS-T; pH 7.4). Incubation with secondary antibodies – Goat anti-Rabbit IgG (Merck EMD Millipore Corp, USA, Cat. No. 12-348) was performed for 1–2 h at room temperature. Afterwards, the membranes were washed three times for 15 min in TBS-T and then visualized. To obtain images of protein bands on the blots, an iBright Imaging System equipped with software version 4.05 and a 9.0 high-resolution Azure Biosystems camera was used. Protein levels were normalized to the total protein content using α -actin as a loading control.

Determination of the antioxidant activity of flavonoids. The antioxidant activity of Hsd and Hst was evaluated based on their ability to scavenge free radicals in a stable methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM). The test samples were incubated with the substrate in the dark at room temperature for 30 min. The flavonoid samples were sequentially extracted using aqueous and methanolic solvents with a Soxhlet apparatus. Serial dilutions of flavonoid solutions at various concentrations (5–550 µg/mL) were prepared. The flavonoid solutions were added to a 0.1 mM methanolic DPPH solution at a ratio of 3:2. Aqueous solutions of ascorbic acid with corresponding serial dilutions served as the standard reference compound. The reduction of the DPPH[•] radical to DPPH-H was monitored spectrophotometrically by measuring changes in solution absorbance at 517 nm. Antioxidant activity was calculated as the percentage of inhibition at flavonoid concentrations that inhibited 50 % of DPPH radicals (IC₅₀); convert IC₅₀ to molar concentration:

$$IC_{50} (\mu M) = \frac{IC_{50} (\mu g / mL) \cdot 1000}{M(g \cdot mol^{-1})};$$

where, A control – a mixture of methanol and DPPH solution; A sample – a sample extract and DPPH solution.

All measurements were performed in triplicate, and mean values were used for analysis.

Determination of β-glucosidase activity of A 549 cells. β-Glucosidase activity was determined by the amount of hydrolyzed substrate para-nitrophenyl-β-D-glucopyranoside (pNPG) to para-nitrophenol (pNP) in the final conversion to 1 mL of lysate. To standardly prepared lysates (50 µL), was added the same volume of 10 mM pNPG in 50 mM sodium acetate buffer (pH 5.0). The mixture was incubated at 37 °C for 60 min. To stop the reaction and convert pNP to a colored form, 100 µL of 2 M NaOH was used as a stop reagent. The optical density was measured spectrophotometrically at 405 nm, and β-GA activity was calculated using a calibration curve generated from known concentrations of p-nitrophenol (pNP). The calculation of enzymatic activity was performed taking into account the formation of 1 nanomole of pNP per 1 min per 1 mL under standard conditions according to the formula:

$$\beta\text{-GA} \frac{(nM pNP / min/mL)}{=} \frac{[(A_{\text{sample}} - A_{\text{blank}}) / Kn_{pNP}] \cdot V_{\text{total}}}{t \cdot V_{\text{sample}}};$$

where, A_{sample} – absorbance of sample measured at the appropriate wavelength (405 nm); A_{blank} – absorbance of blank measured at the appropriate wavelength (405 nm); Kn_{pNP} – correspondence between the optical density (absorbance) of the sample and the molar concentration of the formed p-nitrophenol (pNP), i.e., the concentration of the enzymatic reaction product calculated from the values of the calibration curve constructed using known concentrations of pNP; V_{total} – total reaction volume (mL); t – incubation time (min); V_{sample} – volume of lysate sample used in the assay (mL).

Determination of antimicrobial and antifungal activity of flavonoids. The antimicrobial activity of the tested flavonoids was evaluated against representative bacterial and fungal strains, including *Escherichia coli*, *Streptococcus agalactiae*, and *Candida albicans*, by determining their minimum inhibitory concentrations (MICs) using the broth microdilution method.

Antibacterial assay against *Escherichia coli*. *Escherichia coli* 25922 was cultured in Mueller–Hinton broth (MHB) at 35 ± 2 °C for 16–18 h to obtain an overnight culture. The bacterial suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard and subsequently diluted to achieve a final inoculum concentration of approximately $5 \cdot 10^5$ CFU/mL in each well.

MIC determination was performed in sterile 96-well microtiter plates according to the Clinical and Laboratory Standards Institute (CLSI) guideline M07. Twofold serial dilutions of the tested flavonoids were prepared in MHB to obtain final concentrations ranging from 20 to 3000 μ g/mL. Each well was inoculated with the standardized bacterial suspension and incubated at 35 ± 2 °C for 16–20 h. Sterility controls (medium only) and growth controls (bacterial inoculum without the tested compound) were included in each experiment. Bacterial growth was assessed spectrophotometrically by measuring the optical density at 600 nm. The MIC was defined as the lowest concentration of the compound that completely inhibited visible bacterial growth.

Antibacterial assay against *Streptococcus agalactiae*. *Streptococcus agalactiae* ATCC 13813 was cultured on blood agar plates and incubated at 35 ± 2 °C for 18–24 h under 5 % CO₂ to obtain fresh bacterial colonies. Several isolated colonies were transferred into cation-adjusted Mueller-Hinton broth supplemented with 2.5–5 % lysed horse blood (CAMHB-LHB) and incubated at 35 ± 2 °C for 18–24 h to obtain an overnight culture.

The bacterial suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard and subsequently diluted in CAMHB-LHB to achieve a final inoculum concentration of approximately $5 \cdot 10^5$ CFU/mL in each well.

MIC determination was performed using the broth microdilution method in sterile 96-well microtiter plates according to the CLSI guideline M07. Twofold serial dilutions of the tested flavonoids were prepared in CAMHB-LHB to obtain final concentrations ranging from 20 to 3000 μ g/mL. Each well was inoculated with the standardized bacterial suspension and incubated at 35 ± 2 °C for 20–24 h in an atmosphere containing 5 % CO₂.

Sterility controls (medium only) and growth controls (bacterial inoculum without the tested compound) were included in each assay. Bacterial growth was assessed spectrophotometrically by measuring the optical density at 600 nm.

Antifungal assay against *Candida albicans*. *Candida albicans* ATCC 90028 was cultured on Sabouraud dextrose agar, and the isolated colonies were transferred to Sabouraud dextrose broth and incubated at 35 ± 2 °C for 24 h to obtain an overnight culture. The yeast suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard and further diluted in RPMI 1640 medium buffered with 0.165 M morpholine-propanesulfonic acid (MOPS; pH 7.0) to obtain a final inoculum concentration of $0.5 \cdot 10^3$ to $2.5 \cdot 10^3$ CFU/mL in each well, in accordance with the CLSI M27 guideline.

MIC-2 values were determined by the broth microdilution method in sterile 96-well microtiter plates. Twofold serial dilutions of the flavonoids were prepared in RPMI 1640–MOPS medium to obtain final concentrations ranging from 20 to 3000 μ g/mL. After inoculation, the plates were incubated at 35 °C for 24 h. Sterility controls (medium only) and growth controls (inoculated culture without the tested compound) were included in each assay. Fungal growth was evaluated spectrophotometrically by measuring the optical density at 600 nm.

For *C. albicans*, the MIC-2 endpoint was defined according to CLSI M27 as the lowest concentration of the tested compound that produced a 50 % reduction in growth compared with the drug-free growth control.

All experiments were performed in at least six technical replicates, and the results were expressed as mean $\pm$ standard deviation.

Statistical Analysis. Each experiment was performed at least three times. All data are presented as mean $\pm$ standard deviation (SD). Student's t-test was used to compare data between two groups. One-way analysis of variance (ANOVA) was used to compare multiple groups, and a P value < 0.05 was considered statistically significant. Correlation analysis was conducted using Pearson's test in SPSS for Windows, version 16.0 (SPSS, Inc., Chicago, IL, USA). All analyses were performed using the SPSS statistical software package (SPSS, Chicago, IL, USA). Internal control was α -actin, and the ratio of band density to total α -actin was determined.

RESULTS

Changes in the morphological structure of A549 cells during EMT induced by TGF- β_1 treatment. Treatment of epithelial cells with TGF- β_1 , one of the most potent fibrogenic factors (Chattopadhyay *et al.*, 2022), simultaneously promoted both damage to and transformation of epithelial cells (**Fig. 1**). Most untreated A549 cells and cells treated with flavonoids exhibited the typical morphology of epithelial cells, resembling a cobblestone-like shape, while maintaining tight intercellular adhesion (**Fig. 1A–E**).

EMT induction following TGF- β_1 treatment began at a concentration of 2.0 ng/mL. However, noticeable phenotypic changes were observed after prolonged cytokine exposure (48–72 h), as the cells acquired an irregular spindle-shaped morphology (**Fig. 1F**). The appearance of characteristics typical of the mesenchymal phenotype is consistent with the results of numerous studies (Hazan *et al.*, 2000; Yuksel *et al.*, 2021; Lang *et al.*, 2023), particularly in patients with idiopathic pulmonary fibrosis.

The acquisition of a fibroblast-like phenotype by transformed cells (**Fig. 1F**) was associated with loss of cellular integrity due to disruption of intercellular contacts and a significant ($P < 0.05$) increase in the expression of α -SMA and collagen (**Fig. 4**). It should be noted that cell viability was not significantly reduced ($P < 0.05$) at flavonoid concentrations $< 50 \mu\text{g/mL}$. Only higher doses of flavonoids ($\geq 50 \mu\text{g/mL}$) began to inhibit epithelial cell proliferation, inducing pro-apoptotic-like effects (**Fig. 1C, E**). Treatment with Hsd (10 $\mu\text{g/mL}$) and Hst (5 $\mu\text{g/mL}$) in the presence of TGF- β_1 promoted the restoration of natural cell morphology by suppressing further mesenchymal transformation and restoring the epithelial phenotype (**Fig. 1G–L**).

Higher concentrations of the studied flavonoids significantly inhibited the TGF- β_1 -induced EMT effect through substantial ($P^* < 0.05$) suppression of the expression of extracellular matrix components (collagen I, **Fig. 4**) and the expression of the myofibroblast marker α -SMA (**Fig. 4**), which is responsible for disruption of epithelial tissue integrity.

Among all tested concentrations, under TGF- β_1 stimulation, Hsd ($\geq 30 \mu\text{g/mL}$) and Hst ($\geq 7.5 \mu\text{g/mL}$) significantly ($P^* < 0.05$) modulated the structural organization of the extracellular matrix and markedly ($P^* < 0.05$) reduced the expression of the profibrotic mediator plasminogen activator inhibitor-1 (PAI-1) (**Fig. 4**). PAI-1 is an important regulator of extracellular matrix remodeling and is closely associated with the development and maintenance of the profibrotic and mesenchymal phenotype induced by TGF- β_1 .

Therefore, the observed reduction in PAI-1 expression following Hsd and Hst treatment may indicate attenuation of the TGF- β_1 -induced profibrotic response and normalization of extracellular matrix homeostasis. These changes were accompanied by restoration of the characteristic morphological and functional properties of the cells and re-establishment of epithelial-like features (**Fig. 1G–L**).

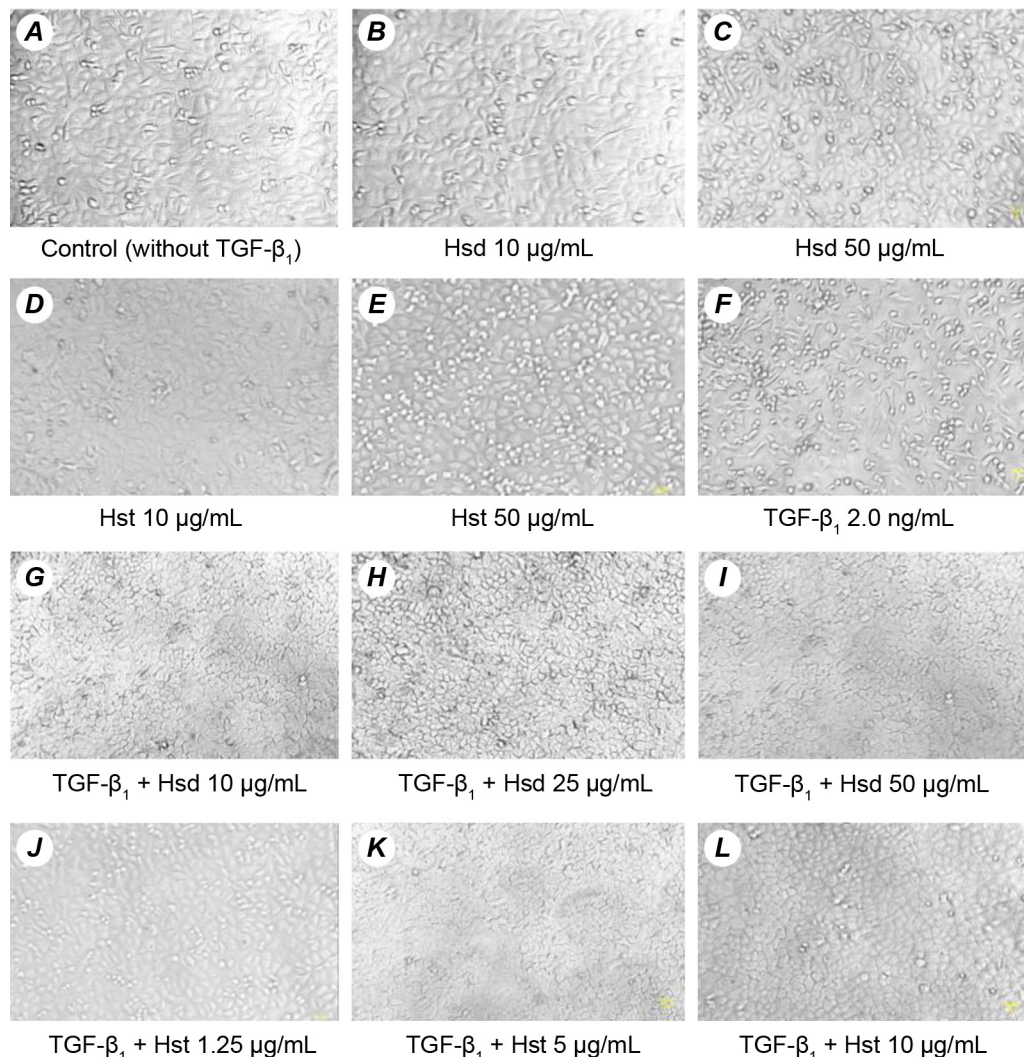


Fig. 1. Morphological changes in A549 lung epithelial cells after treatment with hesperidin and hesperetin in the presence of TGF- β_1 . Cell viability was assessed using light microscopy. Cells were treated with hesperidin and hesperetin together with TGF- β_1 for 72 h. **A** – control cells cultured only in RPMI medium displayed a cobblestone-like appearance with strong intercellular contacts, typical of epithelial morphology; **B**, **D** – treatment with flavonoids alone did not cause noticeable changes in cell morphology; **F** – cells treated with TGF- β_1 exhibited a myofibroblast-like phenotype, characterized by cell elongation and reduced intercellular contacts; **G–L** – Hsd and Hst prevented the morphological changes in epithelial cells induced by TGF- β_1 treatment, helping to maintain epithelial characteristics. **C**, **E** – high doses of Hst and Hsd (≥ 50 µg/mL) without TGF- β_1 began to inhibit epithelial cell proliferation, inducing apoptosis-like changes

Effect of flavonoids on SMAD3/p-SMAD3 expression and downstream proteins. The human alveolar basal epithelial type II cell line A549, owing to its convenient pluripotent properties, was selected as a model for studying molecular markers of EMT. One of the key early molecular markers of TGF- β -induced EMT is phosphorylated SMAD3. Its activation occurs at the initial stages of the signaling cascade and precedes the induction of EMT-associated transcription factors (Snail, Slug, ZEB, Twist) and subsequent changes in the expression of phenotypic markers.

Analysis of the temporal dynamics of p-SMAD3 expression (at 2, 4, and 8 h) revealed differences in its levels. Epithelial cells exhibited high levels of SMAD3 phosphorylation after 2 and 4 h of incubation with TGF- β_1 , reaching a maximum at 4 h (**Figs. 2, 3**), after which a sharp decrease in its expression level was observed. Hst and, in particular, Hsd ($P^{**} < 0.05$), at concentrations $\geq 45 \mu\text{g/mL}$ in the presence of TGF- β_1 , significantly reduced the level of p-SMAD3 ($P < 0.05$) (**Fig. 3**), which was accompanied by subsequent suppression of extracellular matrix (ECM) proteins (collagen and α -SMA expression, **Fig. 4**).

The more pronounced suppression of p-SMAD3 under the influence of Hsd, together with the statistically significant greater reduction ($P^{**} < 0.05$) in the expression of collagen I, α -SMA, and PAI-1 induced by Hst (**Fig. 4**), indicates the complex nature of EMT regulation and possible differences in the mechanisms of action of these flavonoids.

The obtained results suggest that Hsd predominantly exerts its effect through inhibition of the canonical TGF- β /SMAD3 signaling pathway, whereas Hst may additionally or predominantly affect SMAD-independent signaling cascades, including ERK, p38 MAPK, JNK, PI3K/Akt, NF- κ B, and Wnt/ β -catenin. This hypothesis is consistent with current literature data (Hasan *et al.*, 2022; Gaikwad *et al.*, 2023).

In this context, p-SMAD3 should primarily be considered an indicator of activation of the canonical TGF- β /SMAD3 signaling pathway, rather than a direct marker of an already established mesenchymal phenotype.

EMT, as an initial stage of fibrogenesis, is associated with inflammatory signaling and cellular transdifferentiation, which may act independently or synergistically with oxidative stress (Hosawi *et al.*, 2023). Detection of the key mesenchymal markers – collagen I, PAI-1, and α -SMA – revealed no expression under basal conditions (cells without TGF- β_1 and cells treated only with Hsd or Hst) (**Fig. 4**). α -SMA, a marker of epithelial transformation that is incorporated into stress fibers following its expression and, through SMAD3-mediated synthesis of ECM proteins (Gu *et al.*, 2023; Lang *et al.*, 2023), promoted the myofibroblastic differentiation of epithelial cells (**Fig. 1**). The suppression of collagen expression (**Fig. 4**) observed after flavonoid treatment, through attenuation of TGF- β /SMAD3 signaling (**Figs. 2–3**), is potentially associated with a reduction in reactive oxygen species levels (Liu & Desai, 2015). Specifically, a decrease in ROS (Liu & Desai, 2015; Hosawi, 2023) may attenuate the oxidative activation of TGF- β_1 receptors and their subsequent signal transduction.

Normalization of the radical-scavenging capacity results of flavonoids to molar units (μM), despite the substantial difference in the molecular weights of Hsd and Hst (approximately 610.6 and 302.3 g/mol, respectively), allowed their values to be compared correctly and confirmed the more pronounced antioxidant activity of the aglycone. The notably higher antioxidant activity of the lipophilic Hst was confirmed by its lower IC_{50} value in the DPPH assay ($106 \pm 14.3 \mu\text{M}$) compared with hesperidin ($316 \pm 10.7 \mu\text{M}$) ($P < 0.001$; **Table 1**). The relative inhibitory activity of Hst against free radicals ($80 \pm 3.2 \%$) was close to that of ascorbic acid ($85 \pm 15.9 \%$), and the difference between the IC_{50}

values of Hst and ascorbic acid was not statistically significant ($P = 0.14$), indicating the high free-radical scavenging capacity of Hst. The obtained data are consistent with the significant ($P < 0.05$) suppression of PAI-1 expression (**Fig. 4**), a marker associated with TGF- β -induced EMT and oxidative stress.

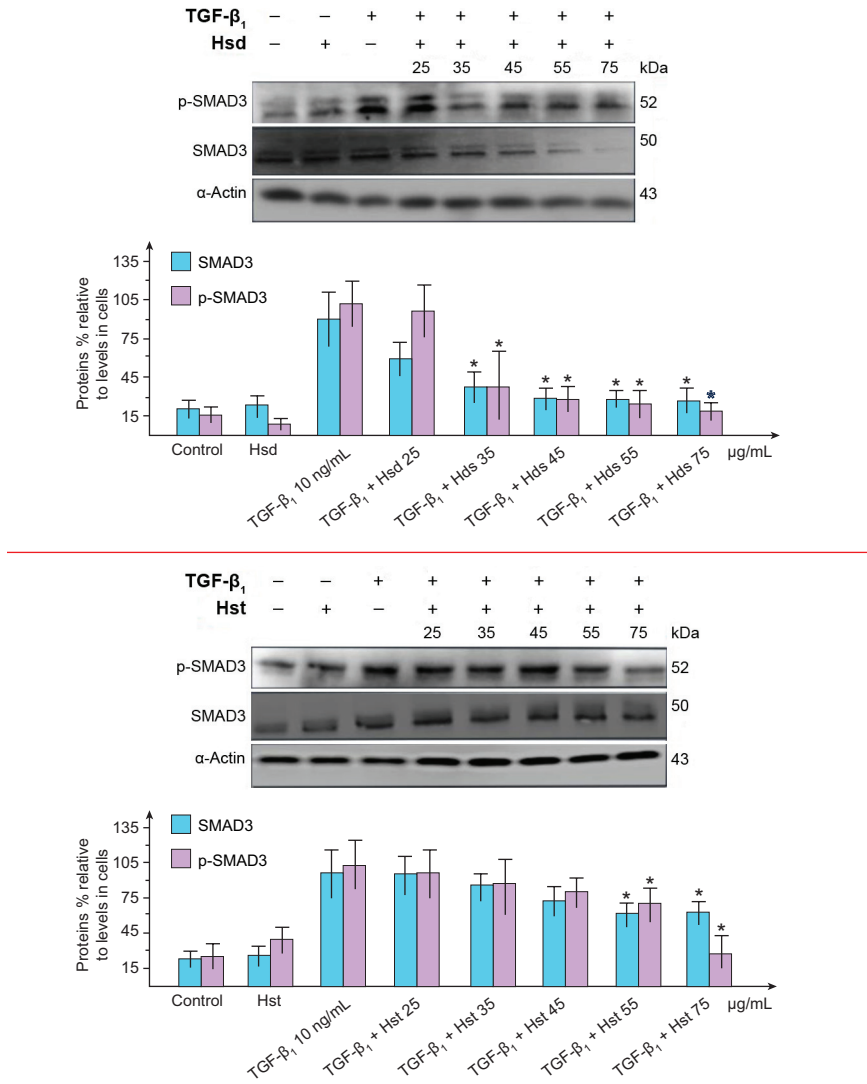


Fig. 2. Western blot analysis of p-SMAD3/SMAD3 expression under the action of flavonoids. The relative expression of p-SMAD3/SMAD3 proteins was measured as described in the “Materials and Methods” section, with quantitative normalization of protein loading to total cellular protein using α -actin. Representative images are presented as immunoblots, and the graphs show quantified specific signals normalized to the corresponding control values and presented graphically ($n = 3$; $P < 0.05$ compared with the control cells without TGF- β_1 and flavonoids, and $P^* < 0.05$ compared with cells treated with TGF- β_1). Each bar in the graph represents the mean value $\pm$ standard deviation. For the detection of phosphorylated SMAD3, a standard lysis buffer containing an inhibitor cocktail was used, as described in the “Materials and Methods” section. During the short-term 2 h exposure experiment, the cells were treated with higher concentrations of TGF- β_1 (10 ng/mL) and flavonoids (25–75 μ g/mL)

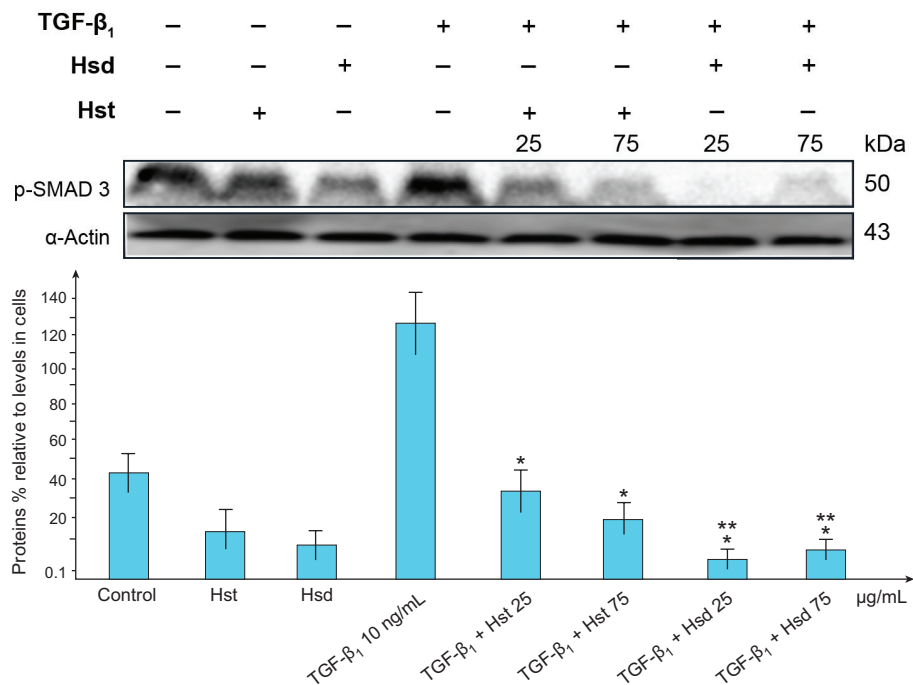


Fig. 3. Comparison of the ability of flavonoids to dephosphorylate p-SMAD 3. The analysis of p-SMAD3 expression in A549 cell lysates over a 4 h period was performed by Western blotting based on the relative expression of p-SMAD3 proteins. Protein loading was quantitatively normalized to total cellular protein using α -actin. Representative images are presented as immunoblots, and the graph showing the quantified specific signals was normalized to the corresponding control values and presented graphically ($n = 3$; $P < 0.05$ compared with cells without TGF- β_1 and flavonoids; $P^* < 0.05$ compared with cells treated with TGF- β_1 ; $P^{**} < 0.05$ compared between hesperidin and hesperetin). Each bar in the graph represents the mean value $\pm$ standard deviation. For the detection of phosphorylated SMAD3, a standard lysis buffer containing an inhibitor cocktail was used, as described in the “Materials and Methods” section. During the 4 h exposure experiment, the cells were treated with higher concentrations of TGF- β_1 (10 ng/mL) and flavonoids (25 and 75 μ g/mL)

Table 1. Indices of antioxidant activity of flavonoids

Compound	Relative*, %	IC ₅₀ , μ M
Ascorbic acid	100	85 $\pm$ 15.9
Hst	80 $\pm$ 3.2 [#]	106 $\pm$ 14.3
Hsd	27 $\pm$ 1.5 ^{###}	316 $\pm$ 10.7 ^{###}

Note: relative, %* – relative inhibitory activity expressed as a percentage relative to the standard (ascorbic acid) at a fixed concentration of 1 μ M. IC₅₀ is given as the mean $\pm$ SD of $n = 3$ independent experiments. Values marked with # in the IC₅₀ column indicate statistically significant differences between the flavonoids and ascorbic acid; ## indicate statistically significant differences between groups ($P < 0.05$, Welch’s t -test). Confidence intervals for IC₅₀ were calculated at the 95 % confidence level

The ability of flavonoids to neutralize free radicals, as demonstrated in our study (Table 1), is often associated with the suppression of PLOD2 and LOXL2 expression (Wei *et al.*, 2021; Brereton *et al.*, 2022) and with HIF-1 α -dependent regulation (Samec *et al.*, 2021; Lotsios *et al.*, 2025). This, in turn, modulates the cross-linking of SMAD3-

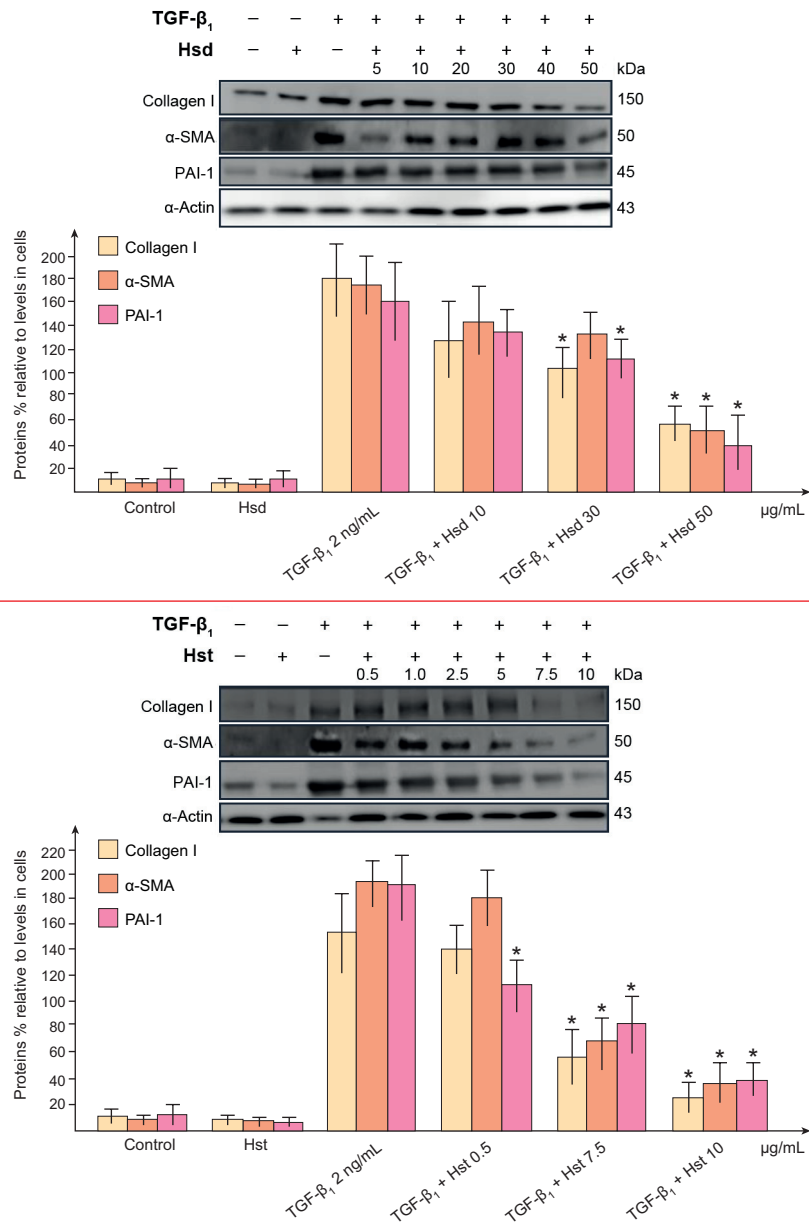


Fig. 4. Western blot analysis of PAI-1 and extracellular matrix protein expression under the action of flavonoids. The expression of extracellular matrix proteins (collagen I, α -SMA) was determined in lysates, and PAI-1 in supernatants, of A549 cells as described in the "Materials and Methods" section, with quantitative normalization of protein loading to total cellular protein using α -actin. Representative images are presented as immunoblots, and the graphs show quantified specific signals normalized to the corresponding control values and presented graphically ($n = 3$; $P < 0.05$ compared with the control – cells without TGF- β_1 and flavonoids, and $P^* < 0.05$ compared with cells treated with TGF- β_1). Each bar in the graph represents the mean value $\pm$ standard deviation

dependent collagen structures (Varadaraj *et al.*, 2017), which constitute 30–70 % of the extracellular matrix (Varadaraj *et al.*, 2017; Hu *et al.*, 2023). Thus, the antioxidant properties of the investigated flavonoids may indirectly reduce fibrotic remodeling of the extracellular matrix not only by suppressing collagen synthesis, but also by disrupting the processes of its post-translational modification and stabilization.

β -GA as a late marker of metabolic transformation of epithelial cells. TGF- β -induced transformation of epithelial cells into myofibroblast-like cells, in addition to increased expression of mesenchymal proteins (**Fig. 4**) ($P < 0.05$), was accompanied by changes in catabolic metabolism associated with EMT development and depended on the duration of exposure. Recent studies by R.-M. Liu and L. P. Desai (2015) and M. Takla *et al.* (2023) have shown that TGF- β signaling may be functionally linked to the activation of Transcription Factor E-box binding protein (TFEB)-dependent biogenesis, whereas TFEB acts as a major regulator of lysosomal hydrolase gene expression and lysosomal catabolic function (Takla *et al.*, 2023).

In this context, the increase in β -D-glucosidase activity observed in TGF- β_1 -induced cells (**Fig. 5**) potentially demonstrates enhanced lysosomal catabolism and metabolic remodeling accompanying EMT development. Cellular β -glucosidase, responsible for the hydrolysis of β -glucosidic bonds, has several isoforms: GBA1 (lysosomal), GBA2, and GBA3 (endoplasmic reticulum and cytosolic) (Sun *et al.*, 2006).

Metabolic remodeling of the cells reduced the total protein content (2.37 ± 0.08 mg/mL) ($P < 0.05$) compared with control cells (2.03 ± 0.11 mg/mL) and cells additionally treated with Hsd (2.50 ± 0.06 mg/mL) or Hst (2.53 ± 0.10 mg/mL) (**Table 2**). Control A549 cells were characterized by relatively low β -GA, at 31.2 ± 3.8 nMpNP / min/mL (**Fig. 5**; $P^* < 0.05$). In contrast, morphologically transformed epithelial cells (**Fig. 1F**) exhibited a statistically significant ($P < 0.05$) increase in β -GA, which was 2.8–3.5-fold higher than that observed in control cells over the 16–72 h period (**Fig. 5**). The increase in β -GA may be consistent with enhanced lysosomal-catabolic activity in the cells and, potentially, with a more pronounced TFEB-dependent lysosomal program during morphological transformation. At the same time, these changes may be associated with increased overall hydrolytic activity of the cells, which could potentially influence the intracellular metabolism of Hsd (**Fig. 6**). In the context of flavanone metabolism, this is important for its conversion into the aglycone form, which demonstrated significantly higher ($P^{**} < 0.05$) biological efficacy in suppressing the expression of mesenchymal markers (**Figs. 2–4**), owing to its increased bioavailability and higher antioxidant activity (**Table 1**).

Temporal analysis demonstrated a progressive ($P < 0.05$) increase in β -GA after 24 h of incubation (61.9 ± 4.5 nMpNP / min/mL), reaching maximal levels during 48–72 h (93.8 ± 2.4 nMpNP / min/mL, respectively). At the early stages of treatment (2–8 h), no significant differences ($P < 0.05$) in enzymatic activity between the experimental conditions were observed (**Fig. 5**), indicating the gradual nature of cellular metabolic adaptation. Treatment of cells with flavonoids in the presence of TGF- β_1 was accompanied by a significant decrease in β -GA (25.6 ± 1.7 nMpNP / min/mL) ($P < 0.05$), simultaneously with a substantial ($P < 0.05$) increase in total protein content (2.50 ± 0.06 mg/mL, **Table 2**). This indicates a mechanism of normalization of the cellular metabolic state and inhibition of TGF- β_1 -induced remodeling.

The elevated enzymatic status of A549 cells under TGF- β_1 stimulation may be considered an adaptive physiological strategy that supports cell survival through activation of a pathologically reprogrammed metabolism, adapted to meet increased energy

and catabolic demands. Our findings are consistent with those of Y. Sun *et al.* (2006) and X. Zhou *et al.* (2017), who reported that excessive β -GA expression contributes to fibroblast-like hyperactivation and tumor growth.

Table 2. Total protein concentration of epithelial cells after treatment with TGF- β_1 and various concentrations of flavonoids

N	Experimental groups	Total protein concentration, mg/mL		
		4 hours	24 hours	72 hours
1	Control	2.31 $\pm$ 0.09	2.33 $\pm$ 0.15	2.23 $\pm$ 0.07
2	Hsd 10 μ g/mL	2.39 $\pm$ 0.05	2.42 $\pm$ 0.09	2.20 $\pm$ 0.05
3	Hst 10 μ g/mL	2.41 $\pm$ 0.12	2.40 $\pm$ 0.12	2.24 $\pm$ 0.06
4	TGF- β_1 2 ng/mL	2.37 $\pm$ 0.08	2.51 $\pm$ 0.13	2.03 $\pm$ 0.11 ^{##}
5	TGF- β_1 +Hsd 10 μ g/mL	2.32 $\pm$ 0.09	2.30 $\pm$ 0.10	2.50 $\pm$ 0.09 ^{#, ##}
6	TGF- β_1 +Hst 10 μ g/mL	2.38 $\pm$ 0.04	2.44 $\pm$ 0.07	2.53 $\pm$ 0.08 ^{#, ##}

Note: data are presented as mean $\pm$ SD (n = 3). Values with # indicate statistically significant differences between variant and control; ## indicate statistically significant differences between groups and TGF (P < 0.05, two-way ANOVA followed by Tukey's post hoc test)

The suppression of “late” EMT markers – PAI-1 (**Fig. 4**) (P* < 0.05) – as well as extracellular matrix proteins (**Fig. 4**) (P* < 0.05) following combined treatment with TGF- β_1 and flavonoids reflects the functional role of β -GA in the metabolic processes accompanying EMT. Although Hst inhibited β -GA more effectively than Hsd (**Fig. 5**) (P** < 0.05), likely due to its higher cellular permeability (Prasanna & Doerksen, 2008) and its ability to interact directly with the enzyme without prior hydrolysis, pathologically altered cells with “sugar deficiency” remain a convenient target for the accumulation and activation of glycosylated molecules and require further investigation.

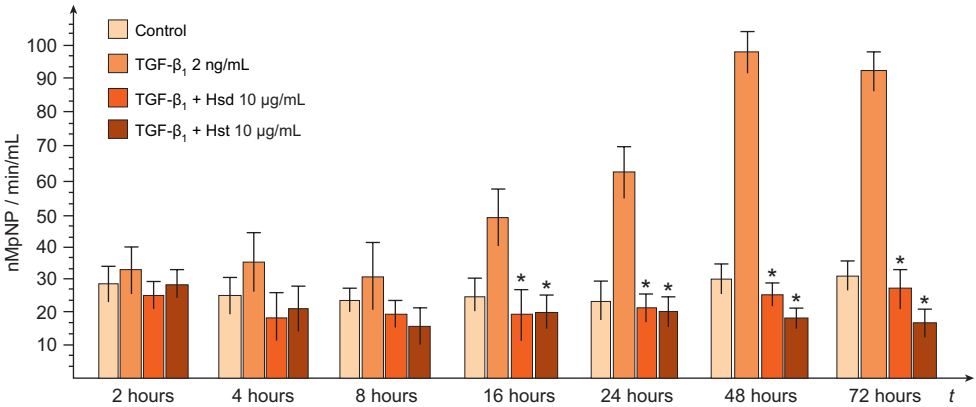


Fig. 5. Temporal dynamics of β -GA activity in epithelial cell lysates after treatment with TGF- β_1 and flavonoids. Data are presented as mean $\pm$ standard deviation (SD) from three independent experiments (n = 3). Statistical significance between hesperidin and hesperetin treatments over time was assessed using Student's *t*-test. Differences were considered statistically significant at P < 0.05 vs. control; *P < 0.05 vs. TGF- β_1 alone; **P < hesperidin vs. hesperetin). Each column represents mean $\pm$ standard deviation

The use of glycosidic flavonoids or semisynthetic glycoconjugates designed according to this principle, combining metabolic selectivity with prodrug-like properties due to the increased target susceptibility of such cells, highlights the pharmacological potential of glycosides (**Fig. 6**).

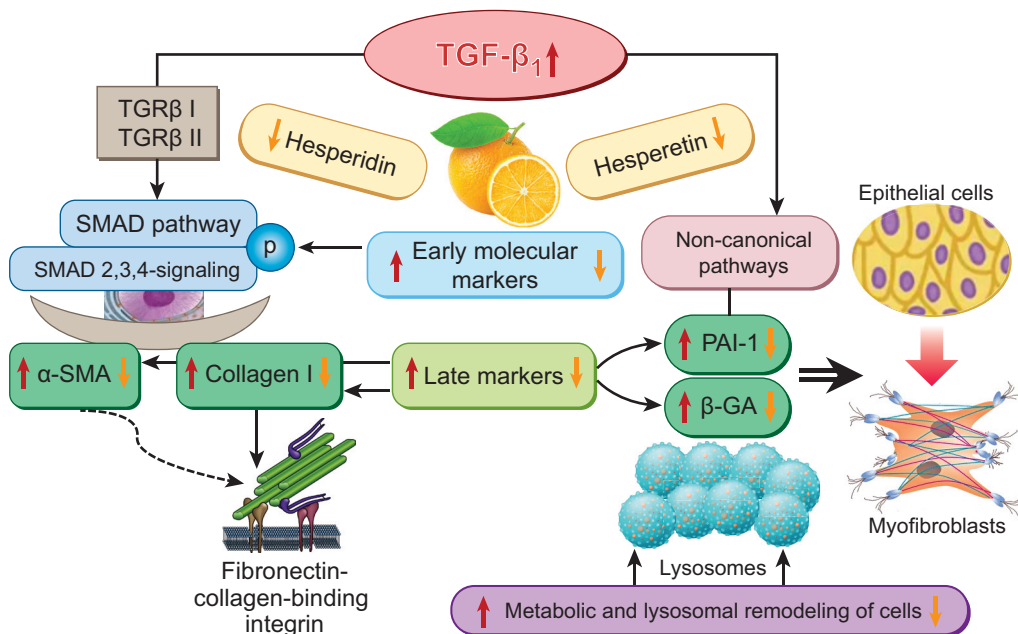


Fig. 6. Effects of flavonoids on markers of TGF-β₁-induced metabolic, lysosomal, and epithelial-mesenchymal remodeling in epithelial cells. TGF-β₁-mediated activation of SMAD3 → initiation of EMT → metabolic, lysosomal, and morphological remodeling of epithelial cells → increased lysosomal β-glucosidase activity and EMT marker expression. Flavonoids attenuate TGF-β₁-induced SMAD3 activation and associated lysosomal changes. Increased lysosomal β-GA may potentially contribute to the hydrolysis/metabolism of Hsd (hypothetical mechanism). Hst more effectively attenuates TGF-β₁-induced EMT-associated changes, including increased α-SMA, collagen I, and PAI-1 expression

Antimicrobial and antifungal activity of flavonoids. The antimicrobial and antifungal activities of the investigated flavonoids were evaluated by determining their MICs against *Candida albicans* ATCC 90028, *Escherichia coli* ATCC 25922, and *Streptococcus agalactiae* ATCC 13813 (**Table 3**). Since microbial infection, biofilm formation, oxidative stress, and TGF-β/SMAD3 signaling are closely associated with epithelial homeostasis and EMT (Heldin *et al.*, 2012; Liu & Desai, 2015), the assessment of the antimicrobial properties of flavonoids is of particular relevance.

The results demonstrated marked differences in the antimicrobial efficacy of hesperetin and hesperidin. Hst exhibited substantially higher antifungal activity against *C. albicans* ATCC 90028, with the MIC value of 39 µg/mL, whereas Hsd showed considerably weaker activity, with the MIC of 512 µg/mL (**Table 3**). This difference may be attributed to the presence of the glycosidic moiety in hesperidin, which can reduce its lipophilicity and membrane permeability compared with the aglycone hesperetin.

In contrast, both compounds displayed weak antibacterial activity against *E. coli* ATCC 25922, with MIC values ranging from 757 to 2845 µg/mL (**Table 3**). The reduced

susceptibility of *E. coli* may be associated with the structural characteristics of Gram-negative bacteria, particularly the presence of an outer membrane containing LPS, which can limit the penetration of polyphenolic compounds (Nikaido, 2003). In addition, efflux systems, such as AcrAB-TolC, may contribute to the extrusion of flavonoids from bacterial cells, thereby reducing their intracellular accumulation.

Table 3. Minimum inhibitory concentrations (MICs) of hesperidin and hesperetin against reference microorganisms

Microorganism	Concentration ($\mu\text{g/mL}$)		
	Hesperidin	Hesperetin	p-value
<i>Escherichia coli</i> ATCC 25922	2845 $\pm$ 204	757 $\pm$ 62.0*	p < 0.05
<i>Streptococcus agalactiae</i> ATCC 13813	1030 $\pm$ 72.6	182 $\pm$ 14.7*	p < 0.01
<i>Candida albicans</i> ATCC 90028	512 $\pm$ 18.3	39 $\pm$ 2.1***	p < 0.001

Note: data are presented as mean $\pm$ standard deviation (SD) from six independent experiments (n = 6). Statistical significance between hesperidin and hesperetin treatments for each microorganism was assessed using Student's t-test. Differences were considered statistically significant at the p < 0.05 level. *p < 0.05, *p < 0.01, ***p < 0.001 compared to hesperidin in the same microorganism

For the Gram-positive bacterium *S. agalactiae* ATCC 13813, Hsd and Hst demonstrated MIC values ranging from 182 to 1030 $\mu\text{g/mL}$ (**Table 3**). The relatively higher susceptibility of *S. agalactiae* compared with *E. coli* may be associated with the absence of an outer membrane in Gram-positive bacteria, which could facilitate the interaction of hydrophobic flavonoid molecules with the cytoplasmic membrane. Citrus flavonoids, by enhancing antioxidant activity (**Table 1**), reduced the expression of EMT- and fibrosis-associated markers (**Figs. 2–4**), and modestly limited the growth of opportunistic microorganisms (**Table 3**). Taken together, these results suggest that flavonoids may modulate processes associated with the TGF- β /SMAD3 signaling pathway that are involved in epithelial–mesenchymal transition, while also exerting moderate antifungal and antibacterial effects against *C. albicans*, *S. agalactiae*, and, to a lesser extent, *E. coli*. Collectively, these observations support the potential therapeutic relevance of citrus flavonoids in conditions characterized by epithelial dysfunction, inflammation, and microbial colonization.

CONCLUSION

The obtained results demonstrate that the citrus flavonoids Hsd and Hst can suppress the expression of early signaling molecules and late markers associated with EMT in an experimental model. Both flavonoids modulate the TGF- β /SMAD3 signaling pathway and attenuate metabolic and lysosomal remodeling associated with EMT. In addition, Hsd and Hst show antimicrobial activity against the opportunistic pathogenic microorganisms tested, although the observed effects were limited. These findings provide new insights into the ability of citrus flavonoids to modulate molecular and signaling processes associated with TGF- β_1 -induced epithelial cell remodeling and EMT. Collectively, these findings support the functional activity of citrus flavonoids as multifunctional compounds with possible relevance to epithelial homeostasis and protection.

AUTHOR CONTRIBUTIONS

Conceptualization, [V.L.; T.M.]; methodology, formal analysis, investigation, data curation, [T.M.; A.O.-R.; L.B.; P.O.; G.B.; M.K.S.; V.L.]; validation, writing-original draft preparation, [T.M.]; writing-review and editing, [T.M.]; visualization, supervision, project administration, [T.M.]; resources; funding acquisition, [T.M.].

All authors have read and agreed to the published version of the manuscript.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that they have no conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

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ВПЛИВ ФЛАВОНОЇДІВ НА МАРКЕРИ МЕТАБОЛІЧНОГО, ЛІЗОСОМАЛЬНОГО Й ЕПІТЕЛІАЛЬНО-МЕЗЕНХІМАЛЬНОГО РЕМОДЕЛЮВАННЯ, ІНДУКОВАНОГО TGF- β , В ЕПІТЕЛІАЛЬНИХ КЛІТИНАХ

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Обґрунтування. Одним із основних ускладнень COVID-19 є легеневий фіброгенез з недостатньо вивченою етіологією, який ініціюється активацією епітеліально-мезенхімального переходу (ЕМП) в альвеолярному епітелії. Беручи до уваги те, що провідним принципом медицини залишається “*primum non nocere*” (“по-перше, не нашкодь”), пошук речовин, що мають широкий спектр терапевтичного потенціалу разом із винятковим профілем безпеки, є актуальним. До таких сполук належать вторинні рослинні метаболіти, серед яких неабияку цікавість останнім часом становлять багатofункціональні цитрусові флавоноїди.

Метою дослідження було дослідити, чи можуть флавоноїди модулювати метаболічне, лізосомальне й епітеліально-мезенхімальне ремоделювання, індуковане TGF- β , за експресією відповідних молекулярних маркерів, а також їхньої антимікробної активності в експериментальній моделі ЕМТ.

Матеріали та методи. Інгібуючий вплив гесперидину та гесперетину на ЕМП досліджували *in vitro* з використанням лінії клітин альвеолярного епітелію людини A 549 типу II. ЕМП індукували TGF- β_1 у концентраціях 2,0 та 10 нг/мл. Морфологічні зміни в клітинах досліджували за допомогою світлової мікроскопії. Експресію мезенхімальних маркерів у клітинних лізатах і супернатантах визначали за допомогою вестерн-блот аналізу. Загальний вміст білка вимірювали за допомогою методу Бредфорда. Антиоксидантну активність флавоноїдів визначали за допомогою DPPH-аналізу, тоді як β -глюкозидазну активність (β -GA) лізатів клітин визначали спектрофотометрично на основі гідролізу pNPG. Антимікробну та протигрибкову активність флавоноїдів досліджували за визначенням мінімальних інгібуючих концентрацій (MIK) щодо *Candida albicans* ATCC 90028, *Escherichia coli* ATCC 25922 і *Streptococcus agalactiae* ATCC 13813 методом серійних мікророзведень у бульйоні. Ріст мікроорганізмів визначали за зміною каламутності середовища після інкубації на відповідних живильних середовищах.

Результати. Гесперидин спричиняв більш виражене зниження рівня фосфорилюваного SMAD3 (p-SMAD3), раннього молекулярного маркера EMT ($P^{**} < 0,05$). Обидва флавоноїди, особливо гесперетин значно ($P^* < 0,05$) знижували експресію мезенхімальних білків (колаген, α -SMA), призупиняючи міофібробластичні диференціації епітеліальних клітин. Окрім того, аглікони, маючи вищу антиоксидантну активність, істотніше ($P^{**} < 0,05$) пригнічував експресію PAI-1 – асоційованого маркера з окислювальним стресом і ЕМП. Флавоноїди модулювали посилений TGF- β_1 лізосомний метаболізм трансформованих клітин, істотно ($P^* < 0,05$) зменшуючи маркер лізосомної перебудови β -GA. Гесперидин та гесперетин виявляли низьку та помірну антимікробну та протигрибкову активність, що потенційно може сприяти підтриманню мікробіологічного гомеостазу та функціональної цілісності епітеліального бар'єру.

Висновки. Отримані результати демонструють здатність цитрусових флавоноїдів (гесперидину та гесперетину) пригнічувати експресію ранніх сигнальних молекул і пізніх маркерів в експериментальній моделі епітеліально-мезенхімального переходу. Гесперидин і його аглікони модулюють сигнальний шлях TGF- β /SMAD3, пригнічують метаболічну та лізосомну перебудову, демонструючи їхній антимікробний потенціал.

Ключові слова: гесперидин, гесперетин, сигнальний шлях TGF- β /SMAD3, лізосомно-метаболічна перебудова, протимікробний і протигрибковий потенціал