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FERULIC & *P*-COUMARIC ACIDS FOR NATURAL COSMETICS: ELASTASE AND TYROSINASE INHIBITION VIA *IN VITRO* AND *IN SILICO* APPROACHS

Luana Luz[1]*, Laís Benvenutti[1], Daniela Valverde[2], Clarice Scliar Sasson[2], Jaciane Lutz Ienczak[1], Acácio Antonio Ferreira Zielinski[1]

[1]*Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, Brazil.*
.[2] *Grupo Boticário, Curitiba, Brazil.*
** Corresponding author's email address: luana.luz@posgrad.ufsc.br*

ABSTRACT

This study investigates the inhibitory effects of ferulic and *p*-coumaric acid on elastase and tyrosinase using a combined computational and experimental approach to elucidate their mechanisms of action and potential for skin care applications targeting aging and hyperpigmentation. Through *in silico* analysis, using molecular docking, it was possible to predict strong interactions between these phenolic compounds and the active sites of enzymes. The results demonstrated that ferulic acid exhibited the highest binding free energy (-5.21 kcal/mol) and the lowest inhibition constant (Ki = 0.15 µmol/mL), indicating potent inhibition of elastase. *In vitro* assays demonstrated that ferulic acid was highly effective against tyrosinase, with an IC_{50} of 0.17 ± 0.01 mg/mL, indicating potent inhibition of melanin production. The *p*-coumaric acid also showed inhibitory activity against tyrosinase (IC_{50} = 2.82 ± 0.07 mg/mL). This evaluation contributes to a deeper understanding of the bioactive properties of these compounds that are found in various plant sources, increasingly seeking applications in natural cosmetic and dermatological products.

Keywords: Enzyme inhibition. Phenolic compounds. Molecular docking. Skin care. Cosmetic products.

1 INTRODUCTION

Skin aging is a complex process, influenced by intrinsic (genetic and chronological) and extrinsic factors (induced mainly by UV exposure) causing photoaging. Both forms of aging contribute to the appearance of wrinkles and sagging skin, in addition to hyperpigmentation caused by UV rays. These processes are often associated with the formation of reactive oxygen species (ROS), which in turn trigger enzymatic processes, including the activation of matrix metalloproteinases (MMPs), which degrades the extracellular matrix (ECM).[1, 2]

Studies using phenolic compounds extracted from plants, such as ferulic acid and *p*-coumaric acid, have demonstrated their potential to delay skin aging processes, due to their antioxidant metabolites, minimizing the activity of free radicals. These compounds also can inhibit degradative enzymes such as elastase and tyrosinase, which are crucial in the degradation of elastin and tyrosine, respectively. [3, 4]

Elastin and collagen are essential components of the extracellular matrix (ECM) in the skin, providing structural support and elasticity. Elevated levels of elastase can compromise skin elasticity and contribute to sagging over time. On the other hand, tyrosinase is responsible for the production of melanin and the excessive melanin production by tyrosinase can lead to hyperpigmentation, resulting in blemishes and accelerated skin aging. The search for inhibitors of skin proteolytic enzymes, such as elastase and tyrosinase, can be obtained from natural sources and may be promising alternatives compared to conventionally used synthetic analogs. [5, 6, 7, 8]

Molecular docking is an *in silico* analysis that investigates, through bioinformatics, the molecular interaction between a small organic molecule (ligand) and biological macromolecules, usually a protein. The objective of molecular docking is to provide a prediction of ligand-receptor binding by analyzing the conformation of the ligand and its position in the active site of the macromolecule. This method allows the evaluation of force fields generated by interactions at atomic levels, including electrostatic interactions, van der Waals forces, and hydrophobic interactions, among others. [9, 10, 11, 12]

In these aspects, this study evaluates the inhibition of elastase and tyrosinase using ferulic acid and *p*-coumaric acid through *in silico* and *in vitro* analyses. These methods complement each other and help to understand the potential effects of these compounds present naturally in plants as active ingredients in cosmetics.

2 MATERIAL & METHODS

The standards Mushroom Tyrosinase (T3824-25KU) and Pancreas Elastase (type I) (E1250), along with the substrates L-DOPA (3,4-Dihydroxy-L-phenylalanine - D9628) and N-succinyl-Ala-Ala-Ala-p-nitroaniline (S4760), were purchased from Sigma-Aldrich® (Steinheim, Germany). Additionally, the standards ferulic acid, *p*-coumaric acid, kojic acid, and EGCG (Epigallocatechin gallate) were also obtained from Sigma-Aldrich® (Steinheim, Germany).

In silico analysis was performed to verify the dermo-cosmetics potential through the ability of target compounds to inhibit enzymes such as tyrosinase and elastase. The 3D conformations of the ligands under evaluation were retrieved from the National Library

of Medicine (pubchem.ncbi.nih.gov) and processed using Open Babel software. Crystal structures of the macromolecules, specifically elastase (PDB ID: 1inc) and tyrosinase (PDB ID: 2y9x), were obtained from the Protein Data Bank (rcsb.org) and prepared using the Discovery Studio program. Subsequently, the macromolecules and candidate ligands were input into Molecular Docking, with specific parameters and active site selections configured. Docking simulations were carried out using the Lamarckian Genetic Algorithm within the Autodock 4 program.[13]

The elastase inhibitory effect assay initially 50 µL of the substrate N-succinyl-Ala-Ala-Ala-p-nitroaniline (1 mM) in Tris-HCl buffer, pH 8.0, was combined with 100 µL of phenolic inhibitors, EGCG (as positive control) and phosphate buffer (as control), at concentrations ranging from 0.2 to 20 mg/mL. Absorbance readings were taken at 410 nm using a microplate reader (Epoch, Synergy-BIOTEK, Winooski, VT, EUA). Subsequently, 50 µL of elastase solution (100 µU/mL in Tris-HCl buffer) and 10 µL of hydrochloric acid were added to the mixture, and absorbance was again measured at 410 nm. The percentage of inhibition was calculated using Equation (1), and the results were expressed as IC_{50} values.[14]

$$Inhibition\ (\%) = \left[1 - \left(\frac{A_2 - A_1}{A_3 - A_0}\right)\right] \quad (1)$$

Where A_0 is the absorbance value of the control assay before the reaction, A_1 is the absorbance of the inhibitor before the reaction, A_2 is the absorbance of the inhibitor after the reaction and A_3 is the absorbance of the control assay after the reaction.

The tyrosinase inhibitory activity was assessed using aliquots of 20 µL of phenolic compounds (ranging from 0.02 to 3 mg/mL), phosphate buffer (for control assay, pH 7.5), and kojic acid (as positive control, 2 to 20 µg/mL) were combined with 10 µL of tyrosinase solution (50 U/mL in phosphate buffer) and 10 µL of phosphate buffer. After pre-incubation at 37 °C for 15 minutes, 90 µL of substrate (L-DOPA, 2 mg/mL) was added, followed by further incubation at 37 °C for 20 minutes. Absorbances were measured at 475 nm, and percentage inhibitions were calculated using Equation (2) and expressed as IC_{50} values.[15]

$$Inhibition\ (\%) = \left[\left(\frac{A_{control} - A_{sample}}{A_{control}}\right) x\ 100\right] \quad (2)$$

Where A_{sample} is the absorbance of acids phenolic and $A_{control}$ is the absorbance of assay using phosphate buffer.

3 RESULTS & DISCUSSION

Through data from molecular docking, we obtained Gibbs free energy (ΔG – kcal/mol) and inhibition constant (Ki – µmol/mL) values for the in silico interactions between macromolecules (enzymes) and ligands (phenolic acids). Additionally, we evaluated the in vitro IC_{50} concentrations of enzymes with both positive controls and phenolic acids, as shown in Table 1.

Table 1 Interactions between macromolecules (skin enzymes) and ligands (phenolic acids) obtained by molecular docking, along with in vitro analyses of elastase and tyrosinase inhibition using phenolic compons.

Bioactive compounds	*in silico*		*in vitro*
	ΔG (kcal/mol)	K_i (µmol/mL)	IC_{50} (mg/mL)
Elastase			
EGCG	-5.38	0.11	1.77[a] ± 0.05
Ferulic acid	-5.21	0.15	1,13[a] ± 0.11
p-coumaric acid	-4.52	0.49	20.05[b] ± 1.20
Tyrosinase			
Kojic Acid	-3.56	2.46	0,02[a] ± 0.01
Ferulic acid	-4.18	0.86	0,17[a] ± 0.01
p-coumaric acid	-3.89	1.40	2.82[b] ± 0.07

The *in silico* analysis revealed strong binding affinities between elastase and the positive control EGCG, presenting the highest binding free energy (-5.38 kcal/mol) and the lowest inhibition constant (Ki = 0.11 µmol/mL) among the tested compounds. Ferulic acid exhibited a binding affinity (**ΔG**: -5.21 kcal/mol and Ki: 0.15 µmol/mL) comparable to EGCG (**ΔG**: -5.38 kcal/mol and Ki: 0.11 µmol/mL) , followed by *p*-coumaric acid (-4.52 kcal/mol and 0.49 µmol/mL). In inhibiting tyrosinase, ferulic acid demonstrated the most effective interaction with a binding free energy of -4.18 kcal/mol and a K_i of 0.86 µmol/mL. Furthermore, *p*-coumaric acid showed efficacy with binding free energy of -3.89 kcal/mol and Ki of 1.40 µmol/mL, overcoming the inhibition of the positive control kojic acid, which presented binding free energy of -3.56 kcal /mol and Ki of 2.46 µmol/mL.

This computational analysis indicates that phenolic compounds have promising potential to inhibit the enzymes elastase and tyrosinase. Figure 1 highlights amino acid residues from the active sites of enzymes involved in molecular interactions with ligands, predicting possible interactions of phenolic acids with amino acids in macromolecules. This approach evaluates key factors such as Gibbs free energy, hydrogen bonds, van der Waals forces, and ionic bonds (π-cation, π-anion, π-π stacking), showing the inhibitory potential of phenolic acids against elastase and tyrosinase. These findings highlight the potential of ferulic acid and *p*-coumaric acid as effective inhibitors of elastase and tyrosinase, suggesting their usefulness in anti-aging and skin-lightening applications.

By the *in vitro* analysis, it was possible to observe that ferulic acid and *p*-coumaric acid showed better results in inhibiting tyrosinase when compared to the IC_{50} concentrations for elastase. Ferulic acid obtained a concentration of IC_{50} = 0.169 mg/mL, a result similar to that reported in the study that investigated ferulic acid in inhibiting melanin in B16 cells, demonstrating that the compound binds directly to the enzyme and inhibits the action of tyrosinase. Research on the inhibition of tyrosinase using extracts from plant

matrices, mainly rich in phenolic compounds, is an expanding field and has demonstrated that extracts enriched with bioactive compounds have inhibitory potential in the production of melanin.

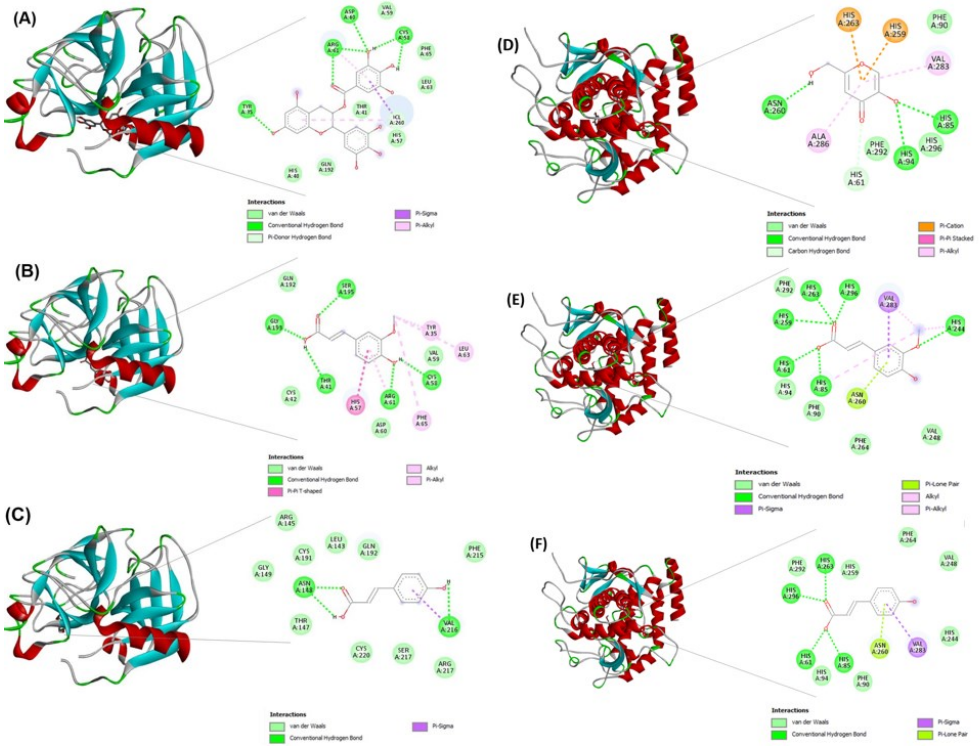

Figure 1 Schematic representation of the interactions between (A) elastase and epigallocatechin gallate, (B) elastase and ferulic acid, (C) elastase and p-coumaric acid, : (D) tyrosinase and kojic acid, (E) tyrosinase and ferulic acid, (F) tyrosinase and p-coumaric acid, obtained by molecular docking.

Furthermore, ferulic acid in vitro analysis demonstrated significant inhibition against elastase, with an IC_{50} value lower than that of the positive control (EGCG), demonstrating that antioxidants such as phenolic acids can prevent aging through the use of these compounds in cosmetics.[16,17,18]

4 CONCLUSION

The results obtained by the *in silico* and *in vitro* analysis indicated the inhibitory effects of ferulic acid and *p*-coumaric acid on the proteolytic enzyme's elastase and tyrosinase, elucidating the mechanisms of action of these phenolic compounds, and evaluating their potential for applications in skin care. Ferulic acid exhibited particularly high binding affinity and potent inhibition of both enzymes. Experimental trials confirmed these predictions, demonstrating the efficacy of ferulic acid against elastase and tyrosinase, with IC_{50} values indicating significant inhibition of enzymatic activity, highlighting the importance of integrating computational predictions with experimental validations in drug discovery and development. The origin of ferulic acid and p-coumaric acid in plant matrices aligns with the growing demand for natural ingredients in cosmetic and dermatological products.

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