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IN SILICO STUDY OF THE PHARMACOLOGICAL TARGETS OF RUTIN AND DETERMINATION OF ITS CHEMICAL FINGERPRINT BY INFRARED SPECTROSCOPY

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+ This work is part of Ernesto Rangel-Sánchez's sabbatical commitment.

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ABSTRACT

Rutin is a naturally occurring glycosylated flavonol widely distributed in plant matrices and associated with several biological activities, including antioxidant and antiproliferative effects. In the present study, an *in silico* analysis was conducted to identify potential pharmacological targets of rutin and to propose possible mechanisms related to its antiproliferative activity, together with experimental evaluation of its cytotoxic effect and determination of its chemical fingerprint by Fourier transform infrared (FTIR) spectroscopy. FTIR characterization confirmed the presence of functional groups characteristic of flavonol glycosides, including phenolic hydroxyl groups, glycosidic linkages, and a conjugated carbonyl system. Cytotoxic activity evaluated in HeLa cells showed a concentration-dependent reduction in cell viability, with a marked inhibitory effect at higher concentrations and an estimated IC_{50} value of approximately 36.4 µg/mL. Pharmacological target prediction using the SwissTargetPrediction platform revealed a preferential interaction profile for rutin with catalytic enzymes, particularly lyases and oxidoreductases, as well as kinases and signaling-related proteins. KEGG pathway enrichment analysis demonstrated significant associations with nitrogen metabolism, acid–base homeostasis, inflammatory pathways, and intracellular signaling involved in cell proliferation. Overall, these results provide combined experimental and computational evidence supporting the antiproliferative potential of rutin and contribute to a better understanding of its molecular mechanisms of action.

KEYWORDS: Rutin; SwissTargetPrediction; KEGG enrichment; ShinyGO; FTIR; Pharmacological targets; Antiproliferative mechanism.

INTRODUCTION

Rutin is a naturally occurring glycosylated flavonol widely distributed in plant matrices and has been reported in the peels of several tropical fruits. This compound belongs to the class of glycosylated flavonols and is characterized by a polyphenolic structure that underlies its diverse biological activities. Scientific interest in rutin arises from the close relationship between its chemical structure and its well-documented antioxidant and metal-chelating properties.[1,2]

In recent years, increasing attention has been directed toward the antiproliferative activity of rutin, particularly its ability to inhibit cell growth in various cancer cell lines, including breast, cervical, colon, and pancreatic cancers.[3–5] These findings suggest that rutin may exert a multifaceted anticancer effect through the modulation of key molecular targets involved in cell proliferation and survival.

Among the main mechanisms of action attributed to rutin is the induction of cell cycle arrest, which has been associated with the downregulation of cyclins (such as cyclin D1) and cyclin-dependent kinases (CDKs), together with the upregulation of cell cycle inhibitors.[6] Additionally, rutin has been shown to promote apoptosis, primarily through the activation of caspase-3 and related apoptotic signaling pathways.[7] Furthermore, several studies report that rutin modulates critical intracellular signaling pathways involved in tumor progression, including PI3K/Akt, MAPK, and NF-κB, which play central roles in cell proliferation, resistance to apoptosis, and inflammatory responses.[8]

Collectively, these molecular mechanisms support the potential of rutin as a bioactive compound with relevant pharmacological activity. In this context, *in silico* approaches represent a valuable strategy to explore the interaction of rutin with specific pharmacological targets, allowing a deeper understanding of its antiproliferative mechanisms at the molecular level.

Therefore, the aim of the present study was to analyze, using *in silico* methodologies, the potential pharmacological targets of rutin and to propose possible mechanisms of action associated with its antiproliferative activity, as well as to determine its chemical fingerprint by Fourier transform infrared (FTIR) spectroscopy.

MATERIALS AND METHODS

Fourier Transform Infrared (FTIR) Analysis

Dry samples were analyzed by Fourier transform infrared (FTIR) spectroscopy using a Cary 630 FTIR spectrometer (Agilent Technologies) to identify the functional groups present. FTIR spectra of the methanolic extract obtained from Annona squamosa peel and a rutin reference standard were recorded as percent transmittance (%T) as a function of wavenumber (cm^{-1}).

Prior to comparative analysis, all spectra were converted to absorbance using the relationship $A = -\log_{10}(\%T/100)$, ensuring linearity with respect to absorbing functional groups. To enable direct spectral comparison, both spectra were interpolated onto a common wavenumber grid spanning 650–4000 cm^{-1} with a step size of 2 cm^{-1}. A mild baseline correction was applied using a rolling median approach to minimize background contributions and instrumental drift while preserving relevant spectral features.

Cytotoxic assay

Human epithelial cells from cervical carcinoma (HeLa) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37 °C in a humidified

atmosphere with 5% CO_2. Cells were exposed to rutin at 0, 0.2, 1, 5, 25, and 70 µg/mL for 72 h. Cell viability was assessed using the sulforhodamine B (SRB) assay, and absorbance was measured using a microplate reader. Results were expressed as mean ± standard deviation (SD) for concentrations tested in triplicate.

Absorbance values were normalized to untreated cells (0 µg/mL), defined as 100% viability. The dose–response curve was generated by plotting percent viability versus the logarithm of rutin concentration. The IC_{50} value was estimated by interpolation between concentrations surrounding the 50% viability threshold; when nonlinear regression was applied, a four-parameter logistic model (4PL) was used.

In Silico Analysis

In *Silico* Pharmacological Target Prediction

Potential molecular targets of rutin were identified using the SwissTargetPrediction web server (http://www.swisstargetprediction.ch/). The canonical SMILES (Simplified Molecular Input Line Entry System) representation of rutin was used as input, and predictions were restricted to Homo sapiens targets. The platform estimates interaction probability by comparing 2D and 3D structural similarity of the query molecule against a library of known bioactive compounds. Predicted targets were compiled and ranked by probability and molecular class (enzymes, receptors, kinases, etc.) for subsequent functional analysis.

Pathway Enrichment Analysis

To assess the biological relevance of the predicted targets, pathway enrichment analysis was performed using ShinyGO v0.85 (http://bioinformatics.sdstate.edu/go/). The list of gene identifiers obtained from the target prediction step was uploaded, and Homo sapiens was selected as the reference organism.

Functional enrichment analysis was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A false discovery rate (FDR) threshold of 0.05 was applied, and only pathways containing 2–5000 genes were considered. The final report was restricted to the 20 most statistically significant pathways.

To simplify visualization and group functionally related pathways, the “Remove redundancy” option was enabled. Results were visualized using dot plots representing fold enrichment, statistical significance (−log10 FDR), and the number of genes involved.

RESULTS AND DISCUSSION

FTIR Chemical Fingerprint

The FTIR spectrum of rutin, expressed in absorbance mode, confirms the presence of characteristic functional groups associated with flavonol glycosides. The broad absorption band in the 3200–3500 cm^{-1} region is attributed to O–H stretching vibrations from phenolic hydroxyl groups, indicating extensive hydrogen bonding. Bands observed around 1650–1600 cm^{-1} correspond to C=O stretching of the conjugated carbonyl group and C=C stretching of the aromatic rings. Additional absorptions in the 1200–1000 cm^{-1} region are assigned to C–O–C and C–O stretching vibrations, characteristic of glycosidic linkages (Figure 1). Overall, the FTIR profile is consistent with the reported chemical structure of rutin and supports its successful identification.

The cytotoxic effect of rutin was evaluated using a sulforhodamine B (SRB) assay in HeLa cells. Rutin treatment resulted in a concentration-dependent reduction in cell viability (Figure 2). Low concentrations (0.2–5 µg/mL) produced moderate effects, maintaining cell viability above 70%, whereas a marked decrease was observed at 70 µg/mL, where viability dropped to approximately 8%. Using untreated cells as the reference (100% viability), the dose–response curve showed that the 50% inhibition threshold was reached between 25 and 70 µg/mL. Based on logarithmic interpolation, the IC_{50} value for rutin was estimated to be approximately 36.4 µg/mL. These results indicate that rutin exerts a significant antiproliferative effect at higher concentrations.

In Silico Pharmacological Target Prediction

The molecular interaction profile of rutin was determined using SwissTargetPrediction. The distribution of predicted target classes (Figure 3) indicated that rutin predominantly interacts with catalytic enzymes. The most representative categories were lyases (26.7%) and enzymes (26.7%), followed by Family A G protein-coupled receptors (20.0%). Other functional categories included oxidoreductases (13.3%), hydrolases (6.7%), and kinases (6.7%).

Among the specific molecular targets with the highest predicted probability, multiple carbonic anhydrase isoforms (CA2, CA4, CA7, CA12, CA9) were identified, which are classified as lyases. Rutin also showed predicted affinity for key enzymes involved in oxidative and inflammatory processes, including aldose reductase (AKR1B1), xanthine dehydrogenase (XDH), cyclooxygenase-2 (PTGS2), and arachidonate 5-lipoxygenase (ALOX5). Predicted interactions were also observed with signaling mediators such as tumor necrosis factor alpha (TNF-α) and ribosomal protein S6 kinase alpha-3 (RPS6KA3) (Table 1). Cyclin-dependent kinase 1 (CDK1) was additionally identified among potential targets; although its predicted probability was lower than that of metabolic enzymes, this finding is consistent with the reported ability of rutin to modulate cell-cycle progression and proliferation.

KEGG Pathway Enrichment Analysis

To elucidate the biological implications of the predicted targets, KEGG pathway enrichment analysis was conducted using ShinyGO v0.85 (Figure 4). The results showed significant associations with metabolic and immune-regulatory pathways. The most significantly enriched pathway was nitrogen metabolism (FDR 2.18E−05), driven by the presence of multiple carbonic anhydrase isoforms (CA2, CA4, CA7). This enzymatic activity was also related to bicarbonate reclamation in the proximal tubule, suggesting a potential role of rutin in modulating acid–base balance, a factor that can influence the tumor microenvironment.

Additionally, strong enrichment was observed in pathways related to inflammation and immune response. Notably, NF-κB signaling and arachidonic acid metabolism were enriched, involving PTGS2 (COX-2) and ALOX5. These pathways are consistent with the regulation of complex inflammatory responses, further supported by enrichment in graft-versus-host disease and allograft rejection pathways, mediated by cytokines such as TNF and IL-2. Finally, enrichment of the cGMP–PKG signaling pathway suggests an additional capacity of rutin to interfere with intracellular signaling cascades.

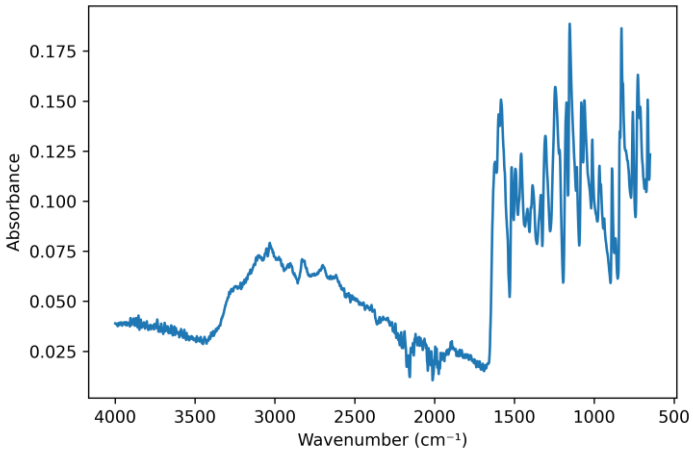

Figure 1: FTIR spectrum of rutin recorded in absorbance mode, showing characteristic bands attributed to phenolic O–H stretching (3200–3500 cm^{-1}), conjugated C=O stretching (~1650 cm^{-1}), aromatic C=C vibrations, and C–O/C–O–C stretching associated with glycosidic linkages.

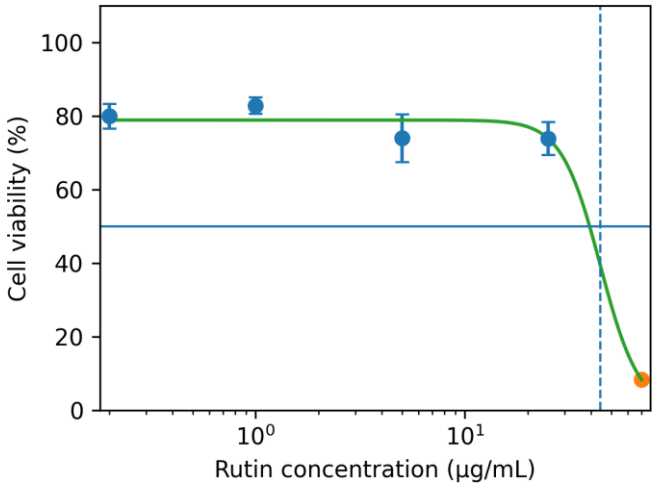

Figure 2: Dose–response curve of rutin in HeLa cells. Cell viability was normalized to untreated control (0 µg/mL = 100%) and plotted against rutin concentration on a logarithmic scale. Data points represent mean ± SD when available. The horizontal line indicates 50% viability.

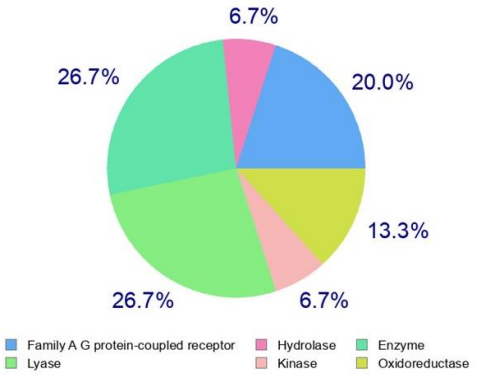

Figure 3: Predicted molecular target profile for rutin. Percentage distribution of target classes obtained from Swiss Target Prediction, highlighting the predominance of catalytic enzymes (lyases and oxidoreductases) among predicted targets.

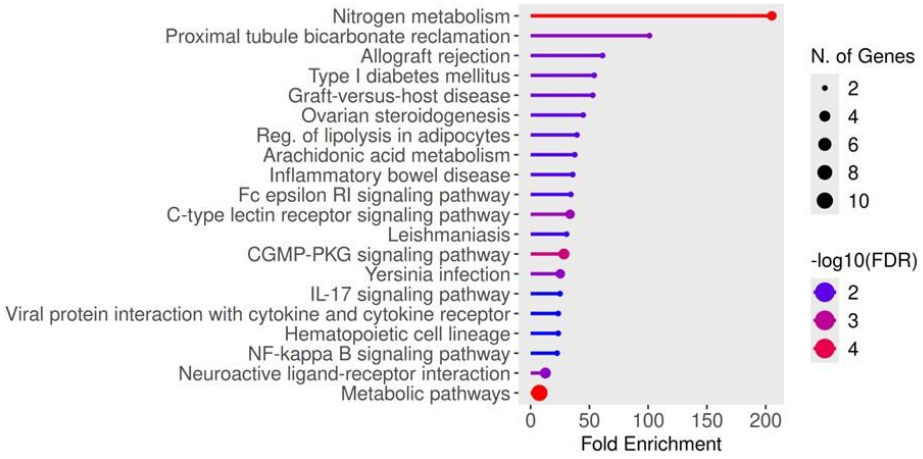

Figure 4: KEGG pathway enrichment analysis of predicted pharmacological targets of rutin (FDR < 0.05). Dot size represents the number of genes mapped to each pathway, and color intensity indicates statistical significance (−log₁₀ FDR).

Table 1: Main pharmacological targets of rutin identified by in silico prediction.

Target	UniProt ID	Target class
Aldose reductase	P15121	Enzyme
Xanthine dehydrogenase	P47989	Oxidoreductase
Cyclooxygenase-2	P35354	Oxidoreductase
Arachidonate 5-lipoxygenase	P09917	Oxidoreductase
TNF-alpha	P01375	Secreted protein
Ribosomal protein S6 kinase alpha 3	P51812	Kinase
Cyclin-dependent kinase 1	P06493	Kinase

CONCLUSIONS

The results of the present study demonstrate that rutin exhibits a complex pharmacological profile characterized by its ability to interact with multiple molecular targets involved in metabolic regulation, inflammatory processes, and cell cycle control. The chemical fingerprint obtained by FTIR spectroscopy confirmed the structural identity of rutin and supported its correct molecular characterization.

Experimental evaluation in HeLa cells demonstrated that rutin induces a concentration-dependent cytotoxic effect, with measurable antiproliferative activity at higher concentrations and an estimated IC_{50} in the micromolar range. These experimental findings are consistent with the predicted interaction of rutin with molecular targets involved in redox balance, inflammation, and cell cycle regulation.

The identification of multiple carbonic anhydrase isoforms, together with the enrichment of pathways such as NF-κB signaling, arachidonic acid metabolism, and cGMP–PKG signaling, suggests that the antiproliferative effects of rutin may arise from multifactorial mechanisms. Overall, these findings highlight the value of integrating experimental and computational approaches and provide a rational basis for future *in vitro* and *in vivo* studies aimed at validating the proposed pharmacological targets and further exploring the therapeutic potential of rutin.

Ethical Statement

This study did not involve human participants or experimental animals. The Hela cell line used is commercially available and was handled in accordance with institutional biosafety guidelines.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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