Natural flavonoids induce dose-dependent cytotoxicity in marine ciliated protozoa parasites through putative mitochondrial mechanisms

J. L. R. Rama[1*], M. Cabaleiro[1], N. Bocija-Villaverde[1], S. Cabaleiro[2], & N. Mallo[1]

[1] Grupo GIBE, CICA, UDC, A Coruña, Spain
[2] CETGA, Ribeira, Spain
* cabaleiro.diaz@gmail.com

Marine ciliated protozoa are metabolically versatile microorganisms that frequently rely on flexible mitochondrial pathways to adapt to fluctuating environmental conditions, including variations in oxygen availability. In this study, we evaluated the cytotoxic effects of several naturally occurring phenolic compounds on *Philasterides dicentrarchi*, a marine ciliate parasite of turbot, causing important economical losses, developing a Trypan Blue based exclusion assay (Vi-CELL BLU, Beckman Coulter). Flavonoids such as quercetin and kaempferol are known to interact with mitochondrial metabolism, including modulation of reactive oxygen species production and interference with electron transport. *P. dicentrarchi* possess an Alternative Oxidase (AOX), which allows metabolic flexibility under stress conditions.

Cells were exposed to increasing concentrations of Quercetin, Kaempferol, Rutin, Gallic acid, and the reference inhibitors Cycloheximide and Chloramphenicol. Among the tested compounds, quercetin displayed the strongest cytotoxic effect, reducing viable cell counts by more than 95% at 100 µM and showing an estimated IC50 of approximately 48.9 µM. Kaempferol and rutin also exhibited significant dose-dependent cytotoxicity, with IC50 values of approximately 31.0 µM and 35.7 µM, respectively. Gallic acid produced moderate effects, while chloramphenicol showed limited but statistically significant cytotoxicity. In contrast, cycloheximide did not produce significant reductions in cell viability under the tested conditions.

The observed cytotoxic effects may involve disruption of mitochondrial respiration. The moderate effect observed with chloramphenicol, which targets mitochondrial-like ribosomes, further supports mitochondrial involvement in the cytotoxic response.

Overall, our results indicate that naturally occurring flavonoids exert significant cytotoxic effects on *P. dicentrarchi*, likely through mitochondrial-related mechanisms. These findings highlight the potential ecological relevance of environmental phenolic compounds in regulating marine protozoan populations of aquaculture importance and suggest mitochondrial metabolism, including AOX-related pathways, as potential targets for further investigation.

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