ULST Timisoara
Multidisciplinary Conference on Sustainable Development
15-16 May, 2025

In vitro evaluation of flavonoid anti-inflammatory activity

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Abstract: Inflammation is a vital immune response to tissue injury or infection. However, when prolonged or uncontrolled, it may lead to the onset or exacerbation of several chronic diseases. Consequently, the discovery and development of safe, effective anti-inflammatory agents remain a major area of biomedical research. In this study, the anti-inflammatory activity of flavonoids Naringin and Chrysin was evaluated, using the bovine serum albumin (BSA) heat denaturation assay. This assay measures the percentage of inhibition of protein denaturation with the flavonoid, which is then compared with a standard anti-inflammatory drug, such as Sodium Diclofenac.

Introduction

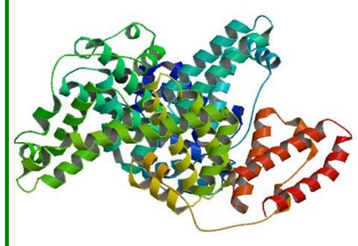


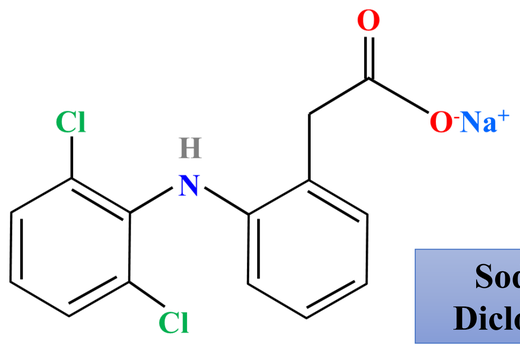
Fig. 1: BSA

Bovine Serum Albumin (BSA) (**Fig. 1**) is a widely used model protein in biochemical assays due to its structural similarities to human serum albumin (HAS) and its well-characterized thermal behavior. In the context of anti-inflammatory screening, BSA denaturation under heat stress serves as a reliable indicator of protein instability, which mimics inflammatory conditions.[1] Moreover, flavonoids are biologically active polyphenolic compounds widely distributed in plants[2] (**Fig. 2**). The plant-derived polyphenolic compounds are known for their ability to inhibit key inflammatory enzymes and factors[3] and stabilize protein structure under thermal stress. In this study, the anti-inflammatory activity of flavonoids (naringin, chrysin) *in vitro* and in a cell-free manner, was evaluated using the bovine serum albumin (BSA) heat denaturation assay.[4] This method assesses a compound's ability to prevent heat-induced protein denaturation. Their ability to form hydrogen bonds and interact with hydrophobic regions of albumin renders flavonoids potential therapeutic tools. The goal of further investigation of structure-activity relationships rides on the potential enhancement of flavonoids therapeutic value and the identification of more potent analogs.

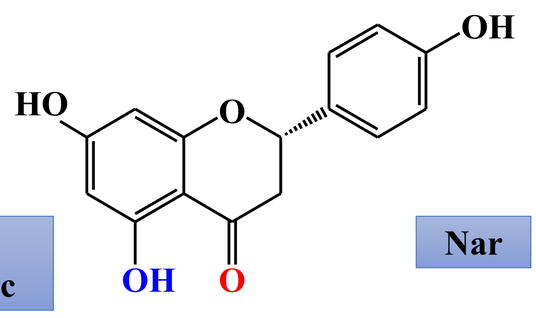
Fig. 2: Flavonoids in plants

Materials and methods

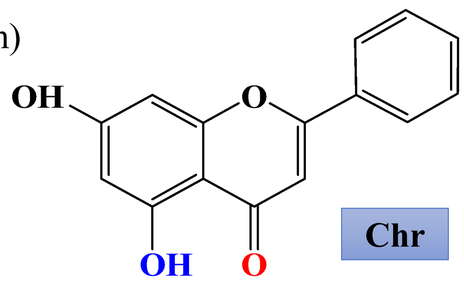
Materials: BSA, PBS, Naringin, Chrysin, Sodium Diclofenac, DMSO
Thermal Denaturation technique: Heat induced 70 °C and 30 min.
Physicochemical Characterization: UV-Visible (Plate Reader: 595 nm & 660 nm)



Sodium Diclofenac

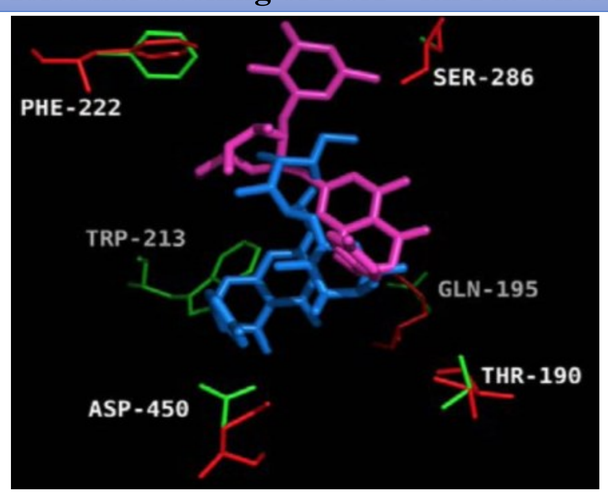


Nar



Chr

Molecular docking of BSA with Flavonoid



Results and Discussion

BSA was adjusted to its isoelectric pH (~5.0) to maximize instability and then it was incubated at 70 °C for 30 min, conditions optimized by monitoring turbidity changes to confirm complete denaturation. The UV-visible assay revealed that both Naringin (Nar) (0.5-50 μg/mL) and Chrysin (Chr) (0.001-20 μg/mL) inhibited BSA (0.4%) denaturation in a dose-dependent manner. Sodium Diclofenac was used as a reference compound (1-80 μg/mL). The IC_{50} values were calculated (**Fig. 3**) and the results suggest that Chrysin exhibits the highest activity, followed by Naringin and Sodium Diclofenac, thus indicating potential anti-inflammatory properties through protein denaturation. The high R^2 values confirms the reliability of the dose-response relationships.

IC_{50} value diagrams

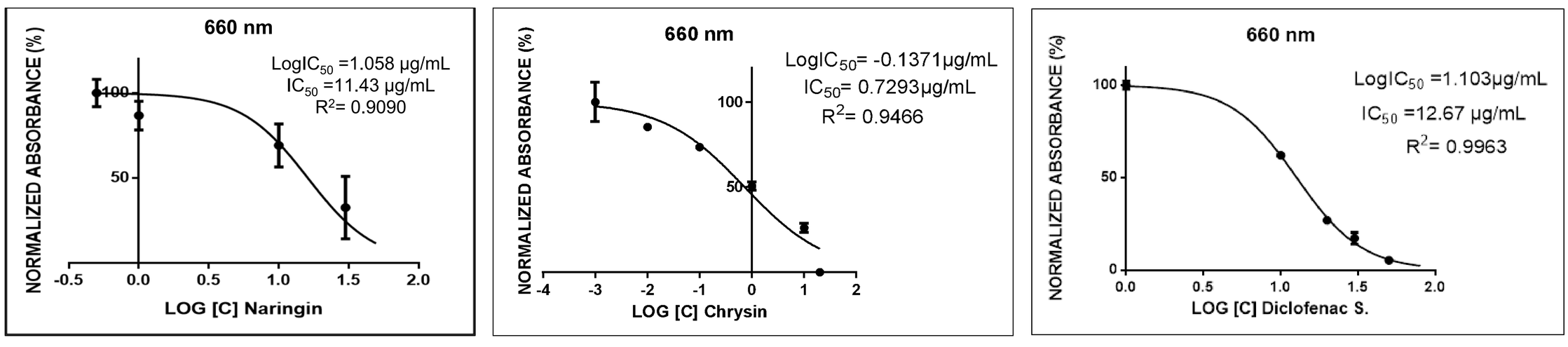


Fig. 3: IC_{50} value diagrams of flavonoid samples (Naringin and Chrysin) and standard drug (Sodium Diclofenac)

Conclusions

- The BSA denaturation assay is highly dependent on experimental parameters such as pH, temperature, and incubation time.
- All tested compounds demonstrated a clear dose-dependent effect, supporting their potential as anti-inflammatory agents.
- The use of natural flavonoids and their analogues may serve as a highly promising alternative to conventional drugs for managing inflammation.

Literature

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