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Ex vivo and *In vivo* Antiinflammatory Evaluations of Modulated Flavanones Solutions

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ABSTRACT

Interest has developed in natural molecules due to their clinically proven effects on skin diseases. Flavanones display several biological activities, and recently have been the focus of studies due to their anti-inflammatory effect. To improve their pharmacological profile four flavanones (**A**, **B**, **C** and **D**), were synthesized by structural modification of one natural flavanone **1** (semi-systematic name: (2S)-5,7-dihydroxy-6-prenylflavanone) extracted from *Eysenhardtia platycarpa*. The hydroalcoholic flavanone solutions (FS) were assayed to investigated their anti-inflammatory effect on two *in vivo* cutaneous inflammation models.

Materials and methods: the topical anti-inflammatory effect of FS were evaluated against models of 12-*O*-tetradecanoylphorbol acetate (TPA) induced mouse ear edema and arachidonic acid (AA) in rat ear edema.

Results: The vinylogous cyclized derivative (flavanone **D**) caused edema inhibition in the TPA- induced models with an inhibition of 96.27 ± 1.93 %; equally effective and potent in inhibiting the mouse ear edema as Indometacine had been. In addition, the AA-induced increase in ear thickness was reduced the most by the topical application of modulated ether (flavanone **B**).

Conclusions: The *in vivo* and histology results suggest that flavanones **B** and **D** are effective as a topical anti-inflammatory agents in inflammatory processes. Thus, this new compounds represents a promising agent for the management of skin diseases with an inflammatory component.

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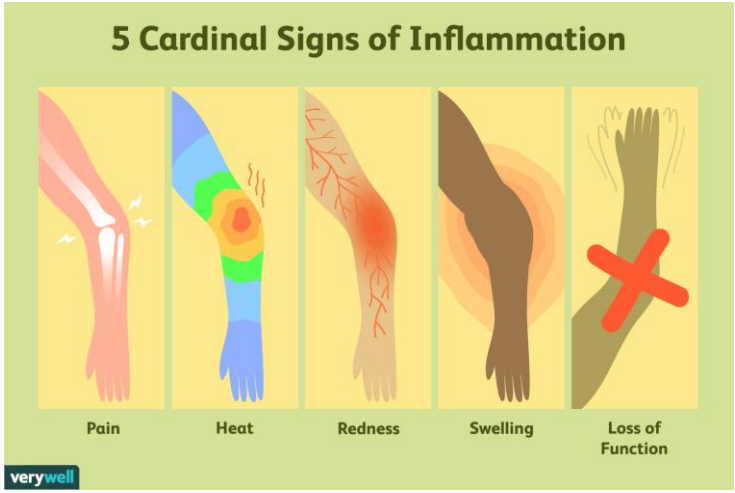
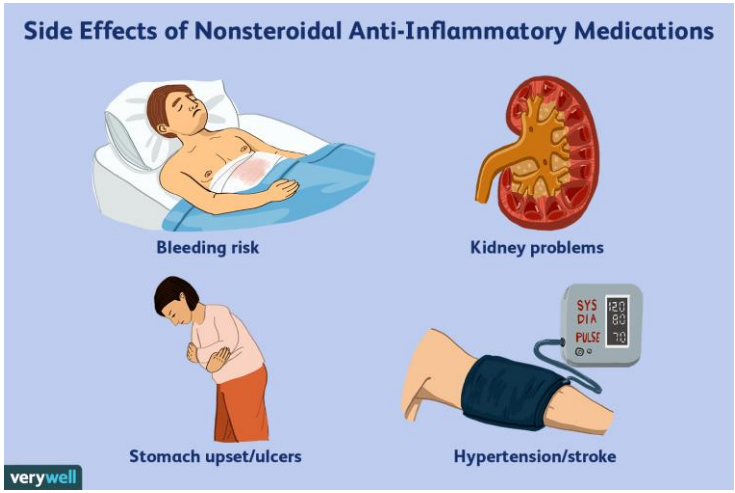
Skin inflammation is one of the most common skin problems.

The most effective route of drug administration where higher concentration of the drug can be accomplished is the topical administration.





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2. Paoletti, T.; *et al. Eur. J. Pharmacol.* **2009**, *620* (1–3), 120–130.

https://www.verywellhealth.com/signs-of-inflammation-4580526

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Natural products

have gathered attention as new anti-inflammatory compounds [3].

Flavanones have been the focus of much research and development due to their several biological activities, included anti-inflammatory effects [4].

Five flavanones were isolated from a methanolic extract of *Eysenhardtia platycarpa* and they showed an anti-inflammatory effect during *in vivo* study [6–8].

3. Singh, M. R.; *et al. Research J. Pharmacognosy and Phytochemistry* **2013**, 5(6): 271-279.
4. Alalaiwe, A.; *et al. Int. J. Pharm.* **2020**, *581* (March), 119-256.
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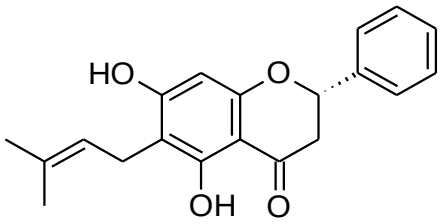
6. Narváez Mastache, J. M.; *et al. Biol. Pharm. Bull.* **2007**, *30* (8), 1503–1510.
7. Pérez Gutierrez, R. M.; *et al. Pharmacogn. Mag.* **2014**, *10* (38), 404–418.
8. Domínguez-Villegas, V.; *et al. Nat. Prod. Commun.* **2013**, *8* (2), 177–180.

https://www.verywellhealth.com/signs-of-inflammation-4580526



1

semi-systematic name:
(2S)-5,7-dihydroxy-6-prenylflavanone

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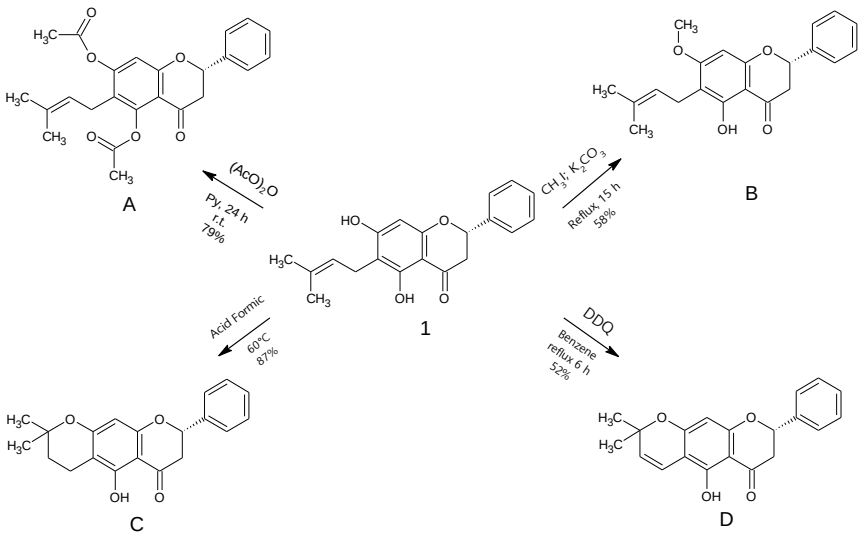
Molecular modification represents one method used by medicinal chemistry for the rational variation of lead compounds with the aim of improving the efficacy, potency and the reducing of undesirable side effects [9].



The **aim** of this research was:

- the *in vivo* anti-inflammatory evaluation of flavanones derivatives (**A**-**D**) in solution using the flavanone **1** extracted from *E. platycarpa* as the starting material (TPA edema mouse and AA (arachidonic acid) edema rat model).
- The histopathology in rat ear was observed.
- We explored the relationship between chemical structure and the therapeutic efficiency of flavanones.

9. Li, S.; *et al. Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 237–250.

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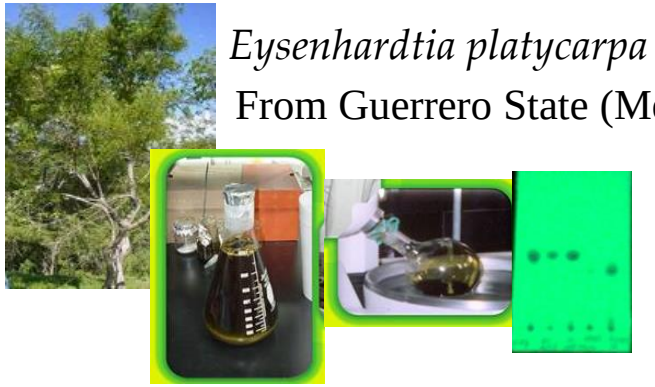
RESULTS AND DISCUSSION

Extraction and Isolation of Plant Material

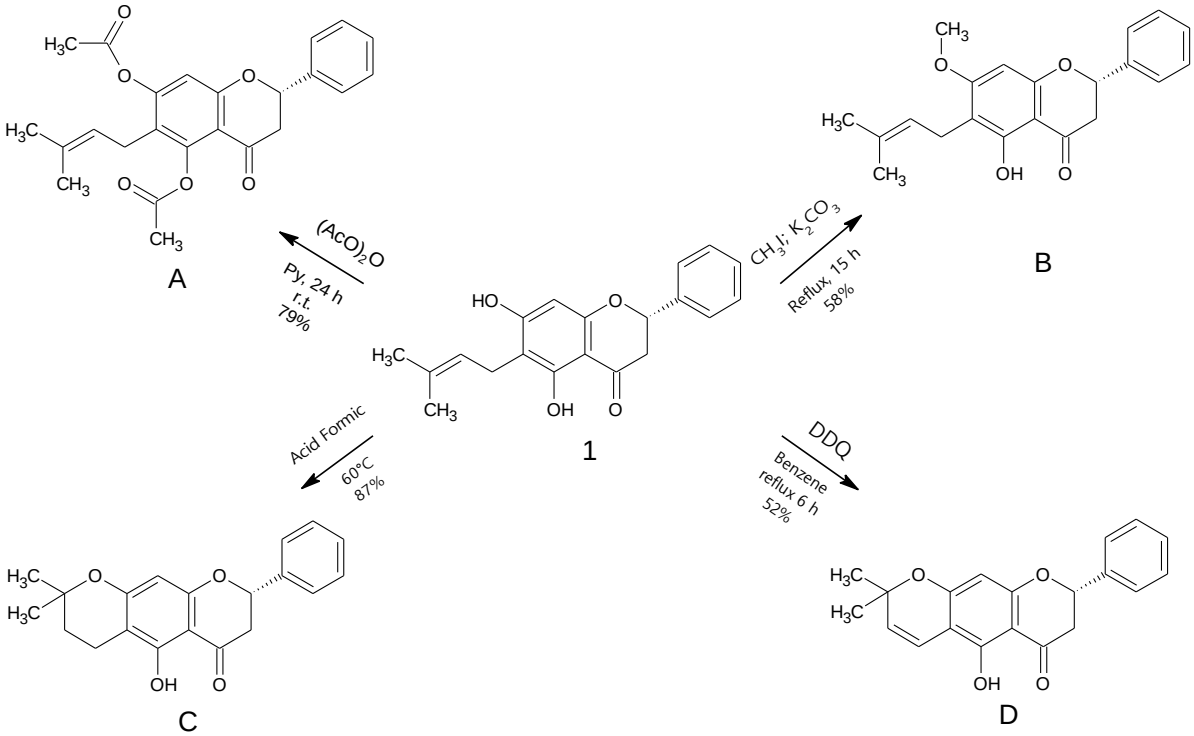


Eysenhardtia platycarpa
From Guerrero State (Mexico).

(100 g) extracted with MeOH (1000 mL)
Isolated by silica gel column chromatography (Flavanone **1**)
Purified by direct thin-layer chromatography (TLC).
Characterized by melting point data and with ^{1}H-NMR.

Domínguez-Villegas, V.; *et al*. *Nat. Prod. Commun.* **2013**, *8* (2), 177–180.

Semi-synthesis from Natural Prenylated Flavanone



Andrade-Carrera, B.; et al. *Molecules* **2017**, *22* (9), 1553.

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Model of Mice Ear Inflammation Induced with TPA

Male Wistar CD-1 mice
(n = 3, 20 to 25 g).

TPA-induced mouse ear edema
(2.5 µg/ 20 µL ethanol per ear, 10 µL each ear side).

Indomethacin (as reference)
Flavanone (**A–D**)/acetone

} both sides of the right ear (1 mg/ ear) with TPA.

Four hours after… inhibition percentage were assessed:

$$Inhibition\ (\%) = \frac{difference\ in\ weight\ of\ ear,control - difference\ in\ weight\ of\ ear,treated}{difference\ in\ weight\ of\ ear,control} \times 100$$

Table 1. *In vivo* anti-inflammatory efficacy after TPA (12-*O*-tetradecanoylphorbol 13-acetate) induced mouse edema. Mean ± SD (n = 3).

Solutions	FS 1	FS A	FS B	FS C	FS D	Indometacin
% Inhibition	66.67 ± 1.55	10.27 ± 0.21	25.69 ± 0.52	40.61 ± 0.81	96.27 ± 1.93	91.35 ± 0.47
Human Skin Retention* (µg/g.cm²)	50.22 ± 7.51	321.52 ± 45.23	381.75 ± 57.26	23.78 ± 5.46	116.14 ± 17.24	ND

* Results of the permeation studies expressed by mean and SD (*n* = 3) reported previously [14].
ND = Not determinated

14. Bustos-Salgado, P.; *et al*. *Biomolecules* **2020**, *10*, 889.

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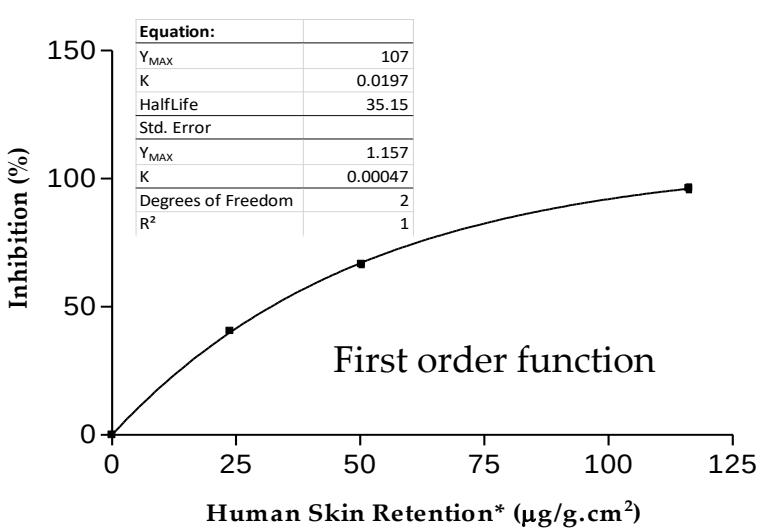
In previous studies [14], the solutions of the flavanone natural **1** and the derivatives flavanones **A**-**D** were evaluated in *ex vivo* diffusional studies in Franz cells using human skin. This was to evaluate their intrinsic permeation and human skin retention (Table 1).



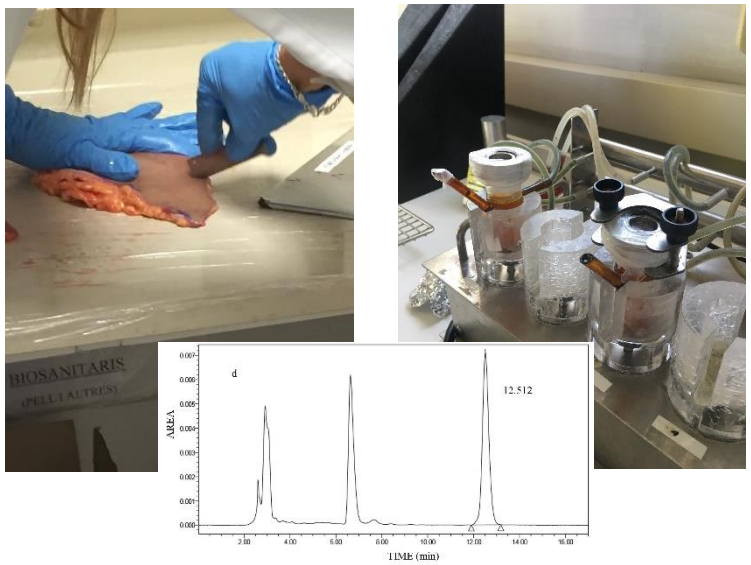


Figure 3. Correlated Function Inhibtion vs Human Skin Retention.

The skin retention results of that study were correlated with the inhibition percentage of mouse edema induced by TPA.

These three flavanones (**1**, **C** and **D**) have similar physicochemical properties, such as area and molecular mass, as well as bond energy.

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14. Bustos-Salgado, P.; *et al. Biomolecules* **2020**, *10*, 889.

Histological Analysis -Arachidonic acid (AA) in rat ear edema model [8]

Adult male Sprague Dawley® rats
(n = 5 /flavanone solution (FS) in EtOH/H_2O (7:3) , 200-240 g).

- 5 mg of AA/1 mL of Phosphate buffered –saline solution.
- 60 µL on both sides of the ears, 20 minutes of exposure.
- 50 µL of (FS **1**, FS **A**, FS **B**, FS **C** and FS **D**) 20 minutes after AA exposition (effected for 20 minutes).
- Ears cut off /rinsed with PBS pH 7.4/ 4 % buffered formaldehyde/embedded in paraffin wax.
- Transversal sections (5 µm) stained with hematoxylin and eosin.
- Observed under a light microscope (Olympus BX41 and camera Olympus XC50).
- Stratum corneum hydration (SCH, arbitrary units AU) corneometer CM825 (Courage & Khazaka electronics GmbH, Köln-Germany).
- Ears thicknesses with a digital micrometer (Wisamic Digital Thickness Gauge 0 - 12.7 mm).
- The edema reduction was calculated by the following equation:

$$\Delta Edema\ reduction = thickness\ after\ treatment - thickness\ before\ treatment$$

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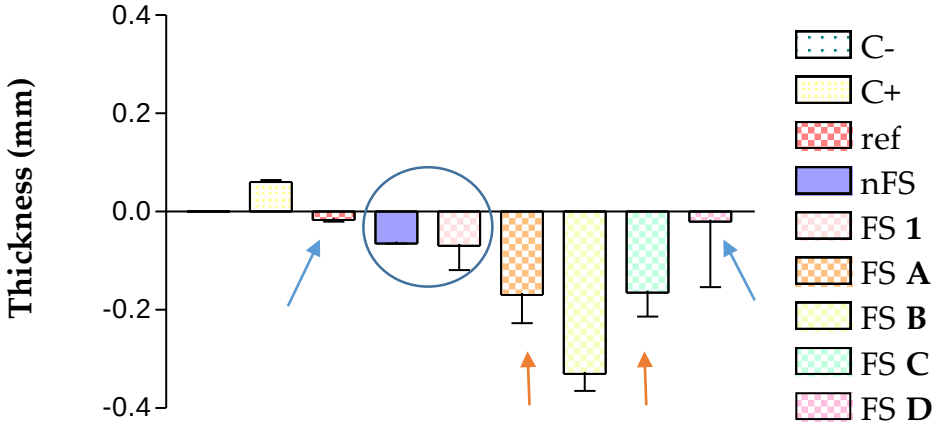
Figure 4. *In vivo* rat model anti-inflammatory response after FS (**A**-**D**) treatment in AA-induced edema model as the increment or decrement of thickness respect to initial conditions. Results are expressed as Mean ± SD (n = 5).



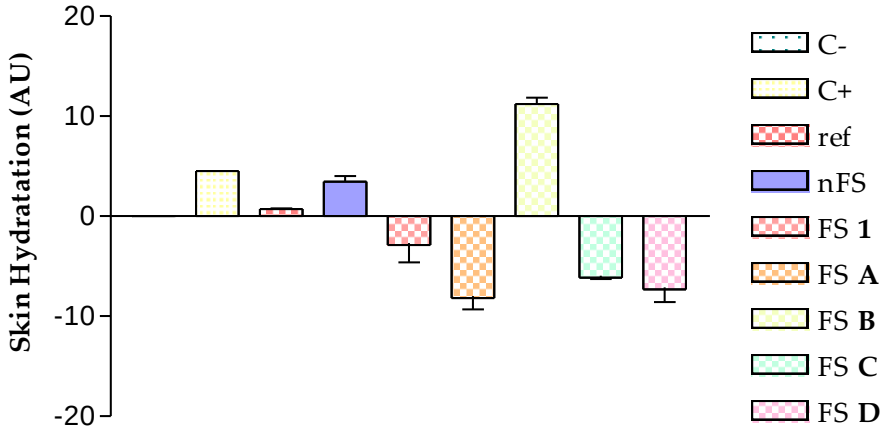
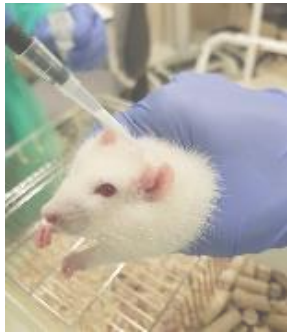


Figure 5. *In vivo* skin hydration after application of after FS (**A**-**D**) in AA-induced rat ear edema as the difference in hydration compared to initial conditions. Results are expressed as Mean ± SD (n = 5).



Where:

C- = negative control,
C+ =positive control,
ref = reference drug,
nFS = ethanol:water,
FS = flavanone solution (**A** – **D**).

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Histological analysis of ear sections for the assessment of the anti-inflammatory effect of the FS. Ears treated with AA (Figure 6) showed a mild inflammation characterized by edema, increased epidermal thickness, and infiltration of polymorphonuclear (PMN) leukocytes.

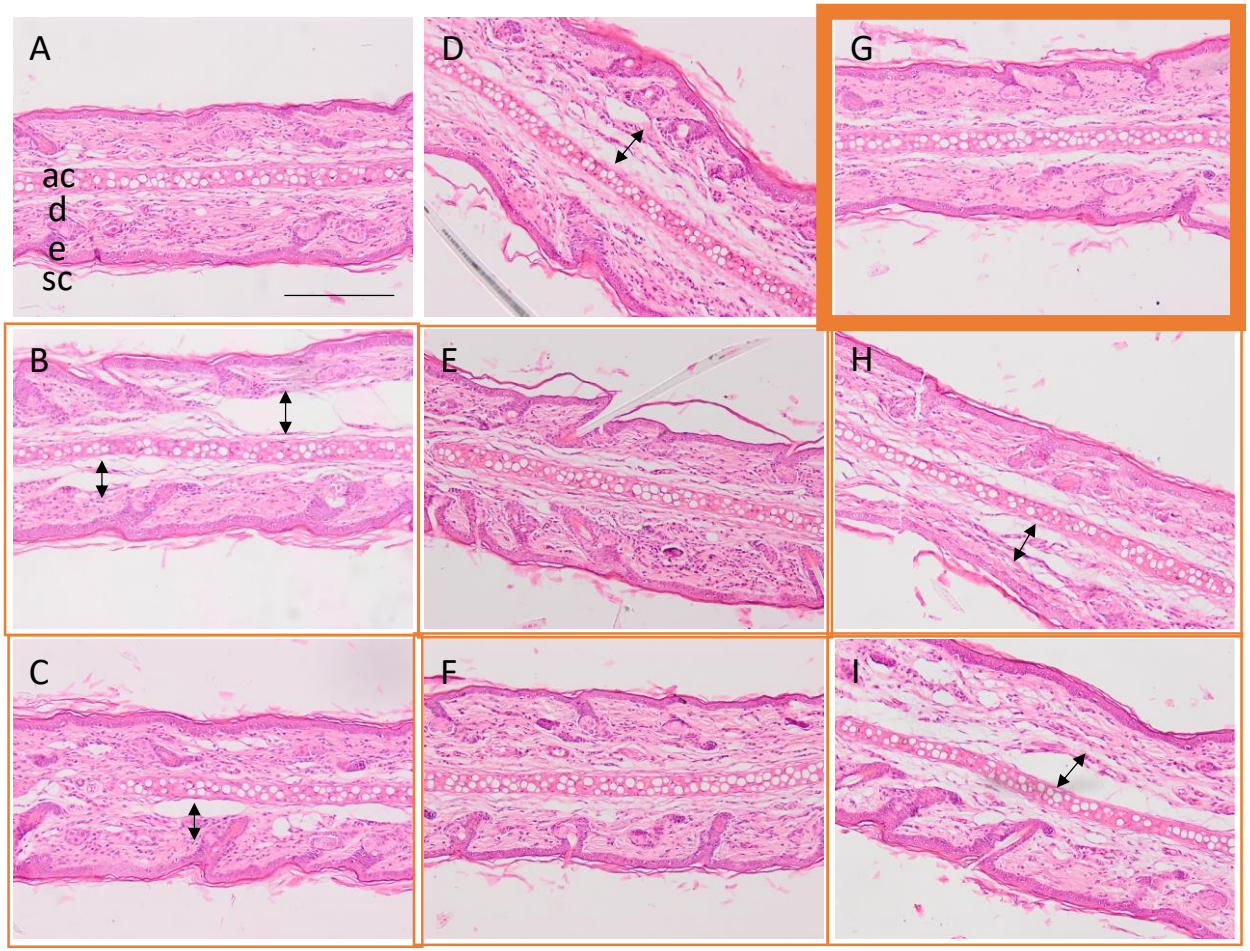

Figure 6. Representative micrographs of rat's ear (x100 magnification). A: control -, B: control +, C: (nFS), D: (ref), E: (FS **1**), F: (FS **A**), G: (FS **B**), H: (FS **C**), I: (FS **D**). e-epidermis, d-dermis, ac-auricular cartilage, sc-stratum corneum. Arrows indicate presence of edema. Scale bar= 200µm

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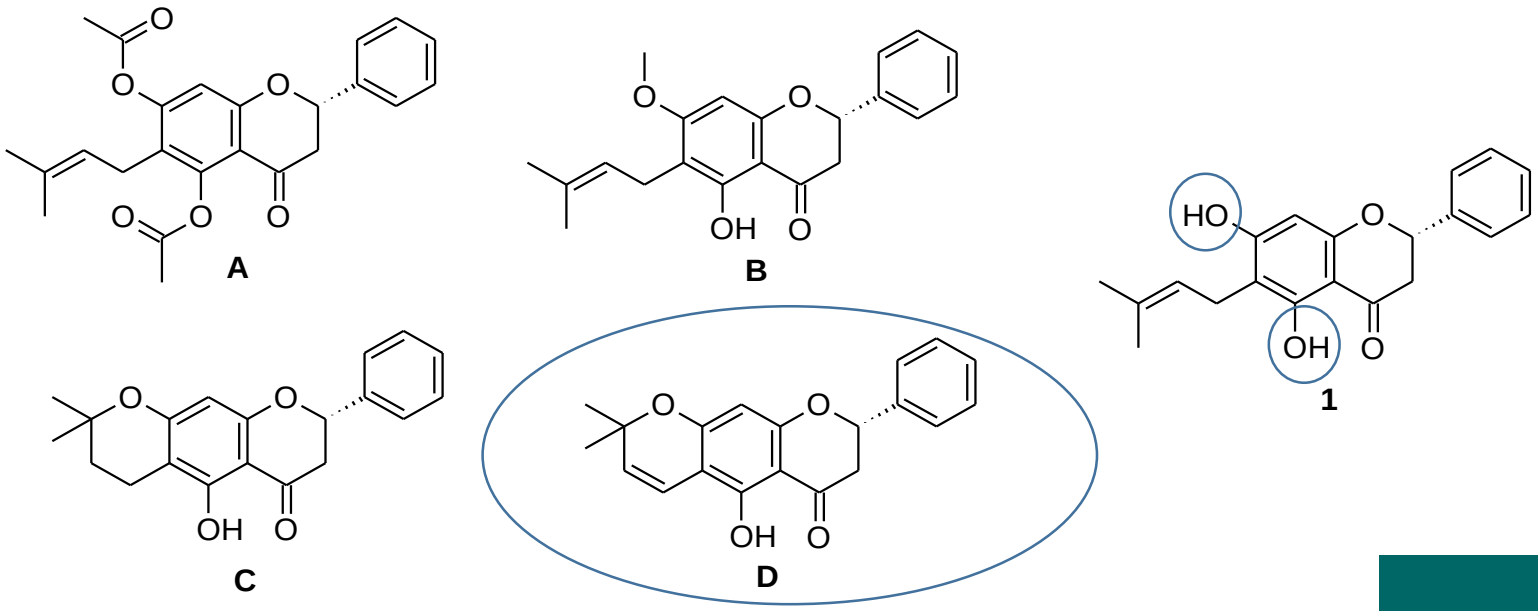
SARs studies of flavonoids revealed that is vital to exhibit the anti-inflammatory action of AA-induced mouse ear edema [15–17].

- A planar ring system and,
- hydroxyl groups at 5- and 7- position of A-ring the inhibition



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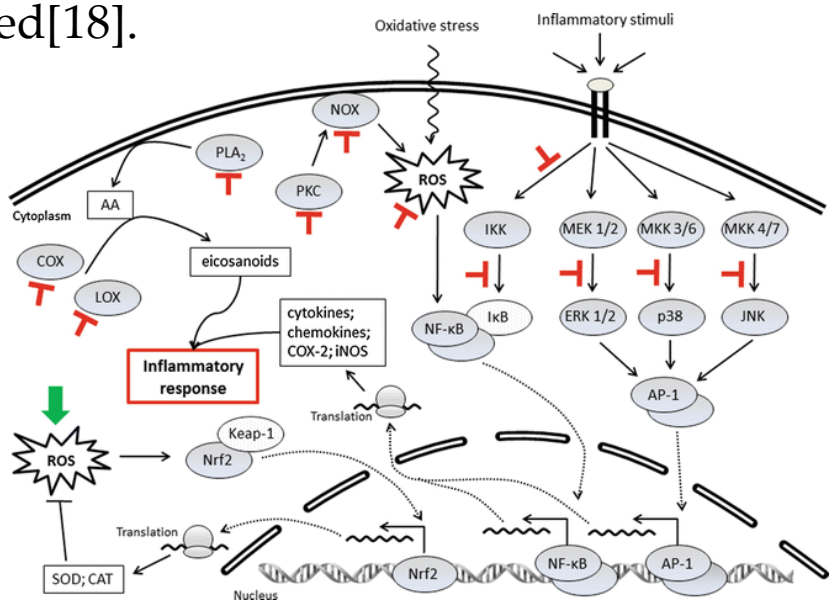
The **working mechanisms** of flavonoids as anti-inflammation agents are still not clearly defined[18].



Dexamethasone is a phospholipase A2 (PLA2) inhibitor more active against TPA-induced than AA-induced ear edema [20].

FS **D** anti-inflammatory activity, was greater against TPA-induced than AA-induced edema. It can be suggested that FS **D** has a similar activity profile to PLA2 inhibitors.

BW755C is a dual COX/LOX inhibitor and it showed a higher anti-inflammatory activity against AA-induced than TPA-induced edema in previous studies [20]. We hypothesize a dual COX/LOX inhibitory activity for FS **B**.

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18. Rauh, L. K.; *et al. J. Ethnopharmacol.* **2011**, *138* (2), 390–397.
20. Escribano-Ferrer, E.; *et al. J. Nat. Prod.* **2019**, *82* (2), 177–182.

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CONCLUSIONS

Conclusions

- The derivation of natural flavanone **1** allowed us to comprehend the importance of molecular structure in an anti-inflammatory action on skin.
- The FS **B** and FS **D** showed better anti-inflammatory efficacy values. Therefore, could and should attract considerable attention for skin inflammatory treatment.
- Future studies can add to current findings leading to better understanding of these flavanones.
- Investigations into the mechanism of action of flavanones must be completed.

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Acknowledgments

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