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# *Humulus lupulus* as a valuable ingredient for cosmetics: assessment of antimicrobial and anti-inflammatory activity

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## Introduction

- *Humulus lupulus*, belonging to the *Cannabaceae* family, is popularly used in traditional medicine for its relaxing therapeutic properties, such as the treatment of insomnia and anxiety [1].
- The inflorescence of *H.lupulus* (the mostly used part of the plant) is responsible for the medicinal character of the plant because it is where the lupulin gland is located, an organ harbouring mainly 15-30% of resins (hard and soft resins), essential oils, polyphenols, among other minority compounds [2].
- The secondary metabolites of *H.lupulus* are known to have a high anti-inflammatory, anxiolytic, antidepressant, antioxidant and antimicrobial potential [3,4].

**AIM:** to evaluate the interest of *H. lupulus* as a cosmetic ingredient, by assessing its bioactivities of interest, *in vitro*.

## Materials & Methods

***H. lupulus***

Flower

Mix

Aqueous extract + Hydrolate

**Chemical characterisation** (Only hydrolates)
- Gas chromatography – Mass spectrometry

**Antibacterial activity (collection strains)**
- *Staphylococcus aureus*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Staphylococcus epidermidis*
- *Cutibacterium acnes*

**Cytotoxicity**
- RAW 264.7 (Macrophages)
- NIH 3T3 (Fibroblasts)

MTT

**Antioxidant effect**
- ROs production (H2DCFDA/Hoechst)

**Anti-inflammatory activity**
- NOs production
- Scavenging activity
- Western blot (COX-2 Protein)

Griess

## Results & Discussion

### Chemical characterisation

***Table 1.*** Chemical characterization of the most abundant compounds found in the mix and flower hydrolates of *Humulus lupulus L.* by gas chromatography-mass spectrometry (GC-MS). The relative amount of each compound expressed in percentage (%) is obtained through the relative area of each compound and the total peak area of the compounds identified in the samples.

| Compound's name | Flower hydrolate | Mix hydrolate (stems, leaves and flowers) |
|---|---|---|
| cis-Linalool oxide | 11.32 | - |
| Linalool | 10.76 | 1.12 |
| Humulenol II | 20.83 | 46.90 |
| Humulene | - | 18.02 |

### Antibacterial activity

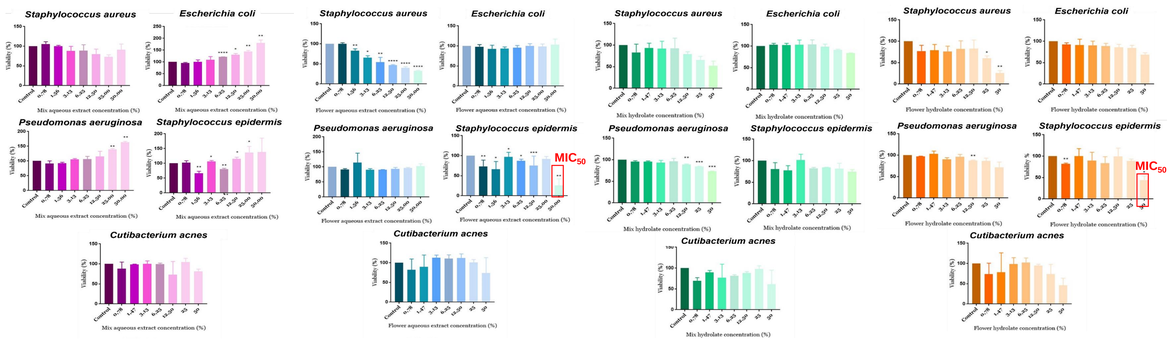


***Figure 1.*** Viability (%) of *S. aureus*, *E. coli*, *P. aeruginosa and S. epidermidis* (after 24 hours) and *C. acnes* (after 72 hours) with contact with different concentration percentile of *H. lupulus* mix (purple) and flower (blue) aqueous extract and mix (green) and flower (orange) hydrolate. Error bar indicate mean ± SD. Significantly P-values are expressed like * p<0.05 ** p<0.01 and ***** p<0,0001 in relation to the control group. The red rectangle shows the mínimum inhibitory concentration ($MIC_{50}$).

- The results show that *H. lupulus* **flower hydrolate** presents higher antibacterial capacity (especially against Gram-positive bacteria) and strong antioxidant capacity, because it significantly reduces the production of ROS following inflammation.

### Cytotoxicity

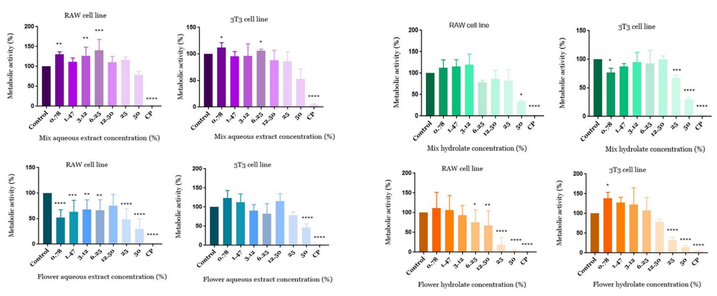


***Figure 2.*** Metabolic activity of RAW cell line and 3T3 cell line after 24 hours of *H. lupulus* mix extract stimuli (purple), flower extract stimuli (blue), mix hydrolate stimuli (green) and flower hydrolate stimuli (orange). Cell lines were stimulated with different concentrations of extract (0.78%, 1.47%, 3.12%, 6.25%, 12.50%, 25% and 50%), with DMEM medium as negative control and with SDS at 1% as positive control (cell death). Error bar indicate mean ± SD. Significantly P-values are expressed like * p<0.05, ** p<0.01 and *** p<0.001 and ***** p<0.0001 in relation to the control group.

### Anti-inflammatory activity

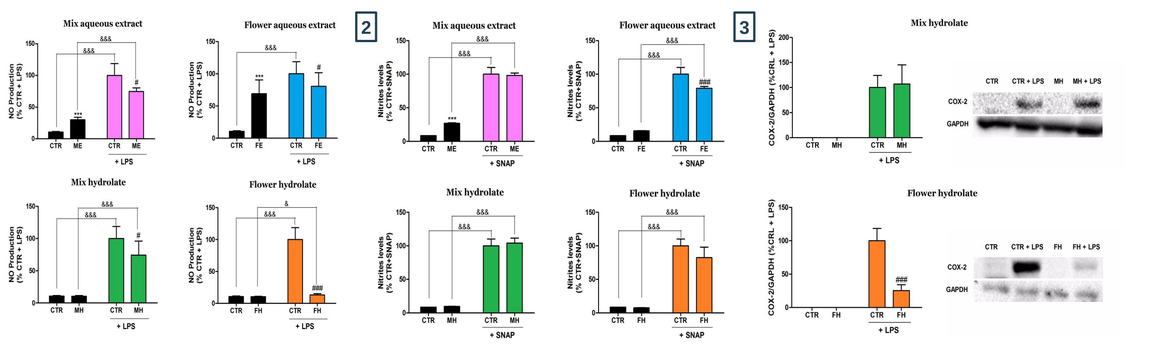


***Figure 4.*** Evaluation of levels of NO production levels by RAW cell line after stimuli with the extracts and with LPS by Griess method (1). Nitrites levels production by the NO donor, SNAP and profile of the extracts to reduce nitrites in the medium using the Griess method (2). Expression of COX-2 protein in Raw cells treated with LPS and with the hydrolates during 24h. The analysis was performed by Western blotting assay. The results were standardized using GAPDH control protein, which means that the same amount of protein was loaded into each well at the time of electrophoresis. Representative immunoblots are showed for the respective graph. Results are expressed as percentage comparatively to control with LPS (3). Error bars indicate mean ± SD. ***, indicates that p<0.001 when compared with control group without LPS. Significantly P-values indicates &&& p<0.001 when compared with respective groups (CRT/extract/hydrolate) without LPS. ### p<0.001 and # p<0.05 when compared with the control group with LPS.

### Antioxidant effect

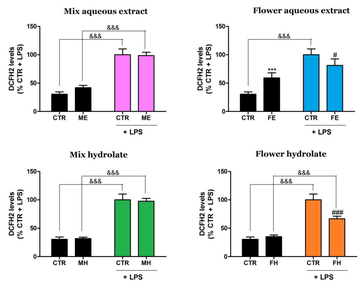


***Figure 3.*** Levels of reactive oxygen species production by RAW cells in the presence and absence of lipopolysaccharide (LPS) with *H. lupulus* substances stimuli by a fluorescence method. Error bar indicate mean ±SD. Significantly P-values indicates &&& p<0.001 when compared with the respective groups without LPS endotoxin. ***, p<0.001 when compared with control group without LPS. ### show that p<0.001 and # p<0.05 when compared to control group with LPS.

- The **flower hydrolate** extract also decreases the production of NO and decreases the expression of COX-2 by LPS activated macrophages, evidencing a strong anti-inflammatory activity.
- The **mix hydrolate** and aqueous extract did not show an antibacterial effect on the studied strains neither significantly altered the metabolic activity of 3T3 fibroblasts and RAW macrophages.

## Conclusions

This study demonstrated that *H. lupulus*, mainly the inflorescence of the plant, has interesting biological activities, specifically anti-inflammatory, antioxidant and antibacterial, that support its possible use as active ingredient for cosmetics products to be in the promotion of skin health.

## References

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