# HYDROXYELENOLIDE

## a new natural product present in extra virgin olive oil

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## Isolation of hydroxyelenolide

During the course of our investigation on the chemical composition of olive tree leaves, we found a new natural monoterpene with an iridoid structure that we have named hydroxyelenolide (**Figure 1**).

***Isolation Method***: The dried and powdered olive leaves were subjected to steam distillation. After removing the precipitate (mainly hydroxytyrosol), the supernatant liquid was extracted with an organic solvent and the crude fractioned by chromatography.

HYDROXYELENOLIDE

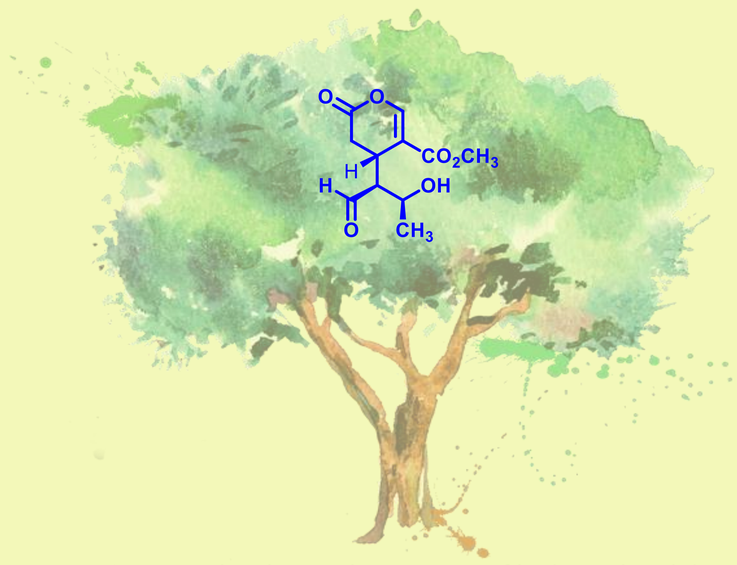


**Figure 1**. The chemical structure of hydroxyelenolide.

## Elucidation of hydroxyelenolide

Hydroxyelenolide structure was elucidated and completely characterized using 1D and 2D NMR techniques. (**Figure 2**).

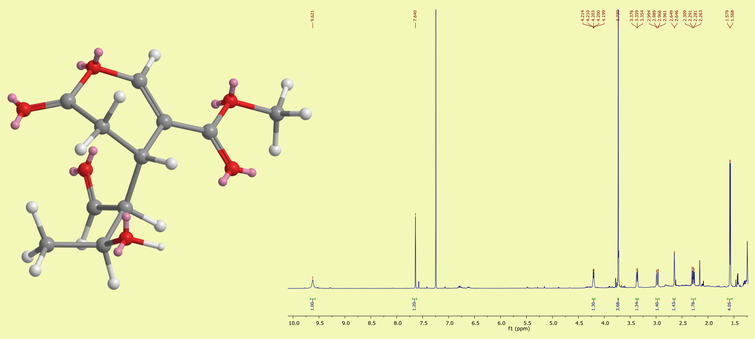

**Figure 2**. $^{1}$H-NMR spectrum and minimum energy conformation of hydroxyelenolide.

## Biological activity

50 μL of a methanolic solution containing the pure compound were added to 2 mL of a MeOH solution of DPPH ($3.6 \cdot 10^{-5}$ M). Absorbance at 515 nm was determined after 30 min in dark, and the percentage of activity was calculated. This new compound has proved to exhibit an antioxidant activity comparable to that of ascorbic acid (**Table 1**).

| Concentration mg/mL | Antioxidant activity of hydroxyelenolide, % | Antioxidant activity of ascorbic acid, % |
|---|---|---|
| **0.002** | 0 | 0 |
| **0.02** | 5,12 | 9,11 |
| **0.4** | 33,33 | 38,99 |
| **1.5** | 48,89 | 45,89 |
| **3.6** | 48,89 | 46,89 |
| **5.5** | 52,47 | 58,47 |
| **6.3** | **70,34** | **62,34** |
| **7.2** | 70,02 | 68,21 |

**Table 1**.Antioxidant activities of hydroxyelenolide *vs.* ascorbic acid.

## Olive oil properties

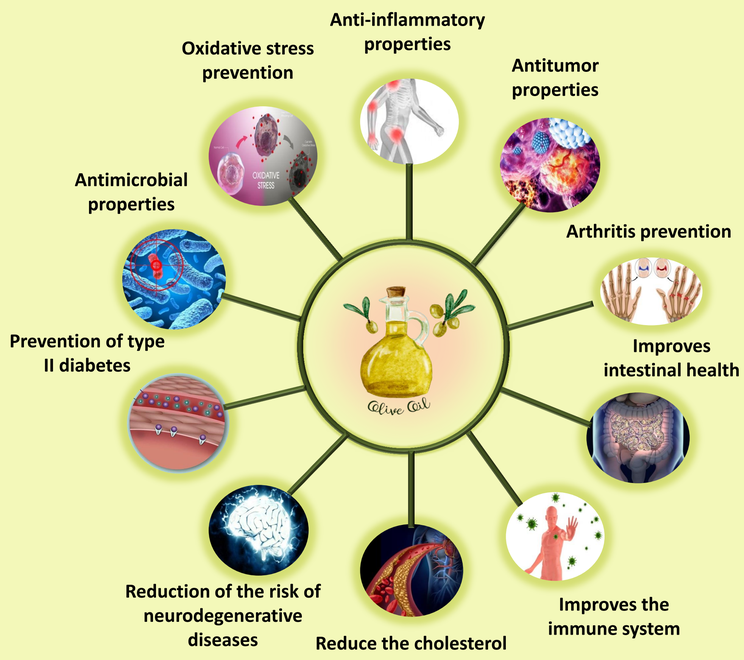


**Figure 3**. Scheme of the beneficial and healthy effects of olive oil.

Extra-virgin olive oil is known to be a key component in the Mediterranean diet and has become globally recognized and appreciated by consumers due to its unique sensory characteristics and because it is responsible of many health benefits (**Figure 3**).[1] These effects are due to its peculiar chemical composition, which includes as its main constituents, unsaturated fatty acids (which are also the major compounds, especially oleic acid) together with an array of biologically active minor compounds such as tocopherols or phenolic compounds, that behave as natural antioxidants.[2]

## Hydroxyelenolide in extra olive oil

- Once we have characterized this new compound found in olive leaves, we looked for a method to detect if this compound was also present in olive oil (**Figure 4**). We analyzed 15 commercial samples of olive oil by a NMR based quantitation method that allowed us to measure its concentration of hydroxyelenolide in olive oil.[3]

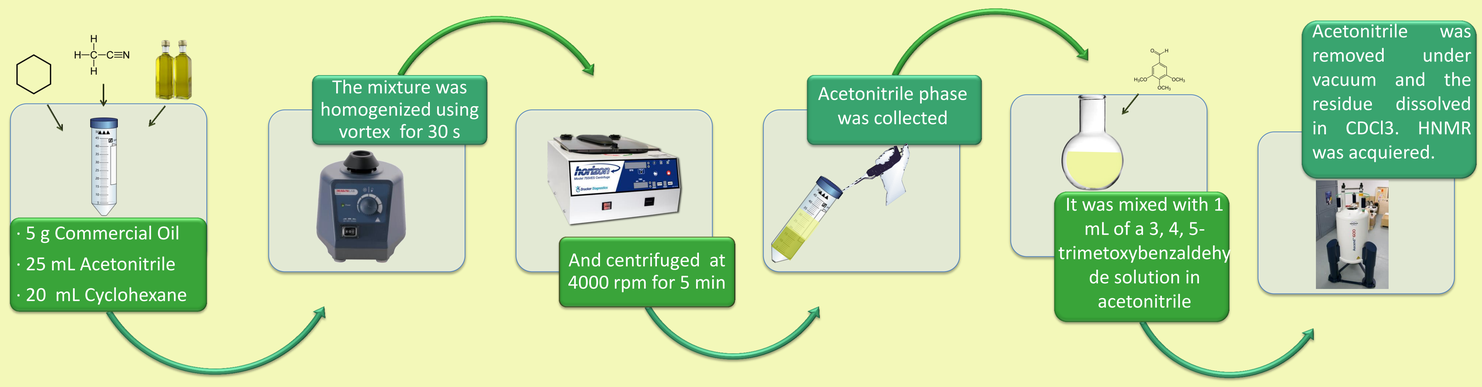


**Figure 4**. Olive oil extraction for analysis.

- Hydroxyelenolide is present only in those oils which have been prepared through cold-press, and its concentration can reach up to 60 mg per $kg^{-1}$ of olive oil (**Figure 5**).

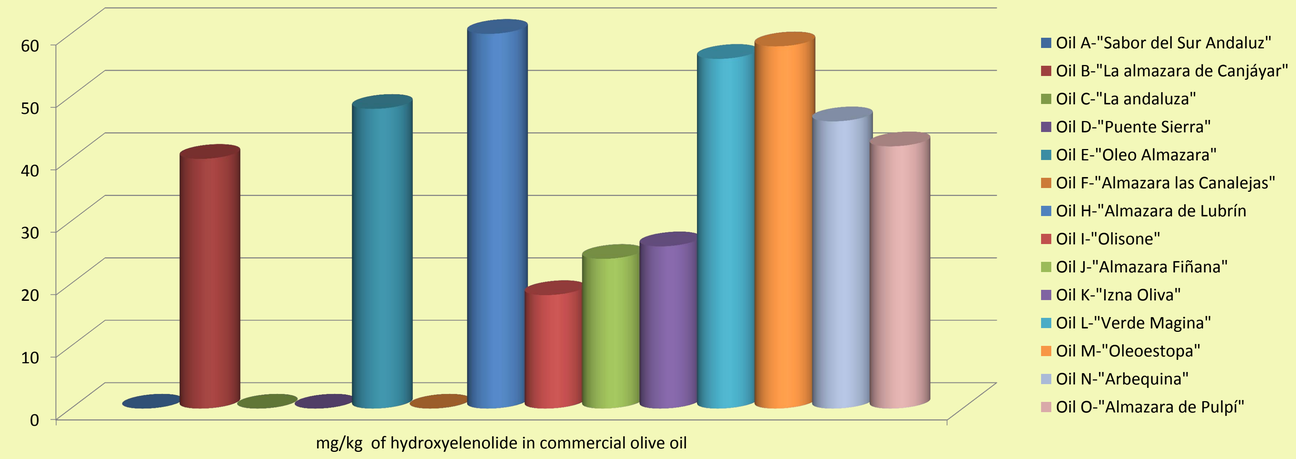


**Figure 5**. Graph of the different commercial olive oils analyzed.

## Conclusion

We have found and elucidated the structure of a new iridoid with health beneficial activities, which is a natural constituent of extra-virgin in olive oil.

[1] Li, X; Wang, S.C. "Shelf Life of Extra Virgin Olive Oil and Its Prediction Models", *J. Food. Qual.* **2018**, *2018*, 1.
[2] Jimenez-Lopez, C.; Cerpena, M.; Lourenco-Lopes, C.; allardo-Gomez, Maria; Lorenzo, J. M.; Barba, F. J.; Prieto, M. A.; Simal-Gandara, J. *Foods,* **2020**, *9*, 1014.
[3] Rigakou, A.; Diamantakos, P.; Melliou, E.; Magiatis, P. *J. Sci. Food. Agric.* **2019**, *99*, 319.

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