Supercritical fluid extraction—based biorefinery of olive leaves residues: A green valorization strategy to obtain terpenoids-rich fractions with potential anti-cholinergic activity.

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1. Introduction

Alzheimer’s disease (AD) is a common neurodegenerative disorder related to older people, accounting for approximately two thirds of all cases of dementia and affecting up to 20% of individuals older than 80 years. AD is characterized by a severe memory loss and cognitive impairments due to a multifactorial neurological pathology, one of them being a progressive decline in acetylcholine levels in the cholinergic systems. Until now, there is no effective treatment for this disease, and a current strategy is to target different factors, such as those related to the high activity of the enzyme acetylcholinesterase (AChE) in the brain.

The olive oil industry has a great economic, commercial and industrial relevance in Spain, being olive leaves one of the main by-products and a promising source of bioactive compounds. Several authors have reported the biological activity of different terpenoids in these residues; among them, the most abundant triterpenoids in olive leaves (derived from oleanane and ursane) have been proved to have an anti-neurodegenerative activity.

Therefore, this work was oriented towards the valorization of olive leaves through the systematic and selective supercritical fluid extraction (SFE) of different families of bioactive terpenoids with potential anticholinergic activity.

2. Results and discussion

A sequential SFE procedure was applied for the isolation of low, medium and high molecular weight terpenoids (C10-C15, C15-C20, C20-C30), using CO2 as pure solvent and different working pressures (from 80-120 bar) at 45°C. Then, extracts obtained at those SFE conditions were characterized by GC-MS and results showed the presence of a wide variety of terpenic structures at different concentration levels. Thus, operating at 120 bar and 45°C during short extraction times (20-40 min), higher levels of monoterpenes (e.g. limonene, anethol, eugenol, thymol) were obtained, while higher molecular weight terpenoids such as caryophyllene, phytol, tocopherols, uvaol, stigmasterol and β-amyrin were obtained at extraction times above 60 min working under the same conditions (45°C and 120 bar).

Figure 1. Supercritical Fluid Extraction equipment
Figures 2a y 2b. Study of kinetic and relative abundance of different SFE fractions from olive leave extracts

Figures 2a and 2b shows the results of kinetic and relative abundance study based on chemical characterization of the different SFE fractions obtained under the above mentioned conditions; different abundance of target terpenoids could be observed, thus showing the selectivity of the proposed strategy.

Subsequently anti-AChE and anti-BChE activities were evaluated and results showed good anticholinergic activity for some of the terpene-rich SFE fractions obtained at 60 and 20 min compared to the global extract obtained at 120 min, indicating that anti-AChE and anti-BChE activities might be related to low molecular weight terpenoids.

Figure 3. Anti-AChE activity

Figure 4. Anti-BChE activity

3. Conclusions
The SFE process optimized in this work allowed obtaining extracts enriched in different families of terpenes according to their distribution in the matrix and volatility. Some of them showed anticholinergic (anti-AChE and anti-BChE) activity, demonstrating the interest of this strategy to help discovering therapeutic approaches from natural compounds that can delay the detrimental effects of AD.

References

Acknowledgements
This work was supported by the project AGL2017-89417-R (MINECO, Spain). G.A.-R. would like to acknowledge the Ministry of Economy and Competitiveness for a Juan de la Cierva-Formación postdoctoral grant. Z.S.M. would like to acknowledge the University of Nariño (Colombia) for financial support.