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Isolation of lupeol from white oak leaves and its anti-inflammatory activity

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ABSTRACT

Lupeol [lup-20(29)-en-2-ol] is found mainly on the surface of plant barks, stems, leaves and fruits waxes. This research explored oaks leaves of several species (*Quercus resinosa*, *Q. grisea*, *Q. laeta* and *Q. obtusata*) as potential source of lupeol. It was extracted from *Quercus* leaves by maceration with $CHCl_3$ at 35 °C, followed by a purification in silica column (normal phase), and using as mobile phase hexane (100%), hexane:ethyl acetate (90:10) and hexane:ethyl acetate (80:20). Lupeol in oak leaves was identified by ^{13}C NMR and quantified by GC–MS. *Quercus obtusata* leaves were an abundant source of lupeol (173.59 μg/g of sample). Anti-cyclooxygenase activity has been used for determining bioactivity of lupeol in this research.

1. Introduction

Nutraceuticals are recognized as biologically active substances present in natural products and foods that when consumed in concentrated form have demonstrated beneficial effects on the health. Plants are the most commonly sources of natural bioactive compounds, which may specifically be helpful in the treatment of certain diseases. *Quercus* species commonly known as oaks have an important distribution in Mexico. Particularly the state of Durango, Mexico, possesses large forest areas with 41 *Quercus* species, from which 22 are whites and 19 are reds (Rosales-Castro et al., 2011). As reported, *Quercus* spp. leaves contain tannins, alkaloids, saponins, cardiac glycosides and steroids (Sánchez-Burgos et al., 2013), and have shown antioxidant, antimicrobial, antitopoisomerase and gastroprotective effects. Infusions of *Quercus* species has been used in folk medicine as treatment for several inflammatory diseases (Maxia et al., 2005).

Inflammatory diseases are one of the major problems in many pathophysiologies such as gastrointestinal disorders. There are many alternatives to treat inflammatory processes, some of which involve the use of nonsteroidal anti-inflammatory drugs (NSAIDs). However, the low enzymatic selectivity of these drugs and the abuse in their consumption cause health problems. This is due to the non-selective inhibition of NSAIDs on cyclooxygenase cytoquines. Therefore, it is justified to explore natural alternatives, which involve the use of bioactive nutraceuticals without side effects as the associated with the prolonged use of NSAIDs (Kumari and Kakkar, 2012).

Among the nutraceutical recognized with major biological potential as anti-inflammatory activity are triterpenes. Lupeol is a pentacyclic triterpene found in many medicinal plants and some fruits (Deyrup et al., 2014; Hernández-Vázquez et al., 2010). This chemical constituent has shown diverse biological effects such as: antioxidant, anti-topoisomerase, antitumor, anti-inflammatory, among other activities (Santiago and Mayor, 2014; Zhang et al., 2015; Kumari and Kakkar, 2012).

Lupeol is distributed along the plant kingdom, and is found in olive fruit, mango fruit, Aloe leaves, Elm Plant, Japanese pear, Ginseng oil (Saleem, 2009) and fig (Santiago and Mayor, 2014) among others, in concentrations between 3 and 880 μg/g of sample. Considering the health benefits that this triterpene provides, the main objective of the present work was to explore new natural sources to

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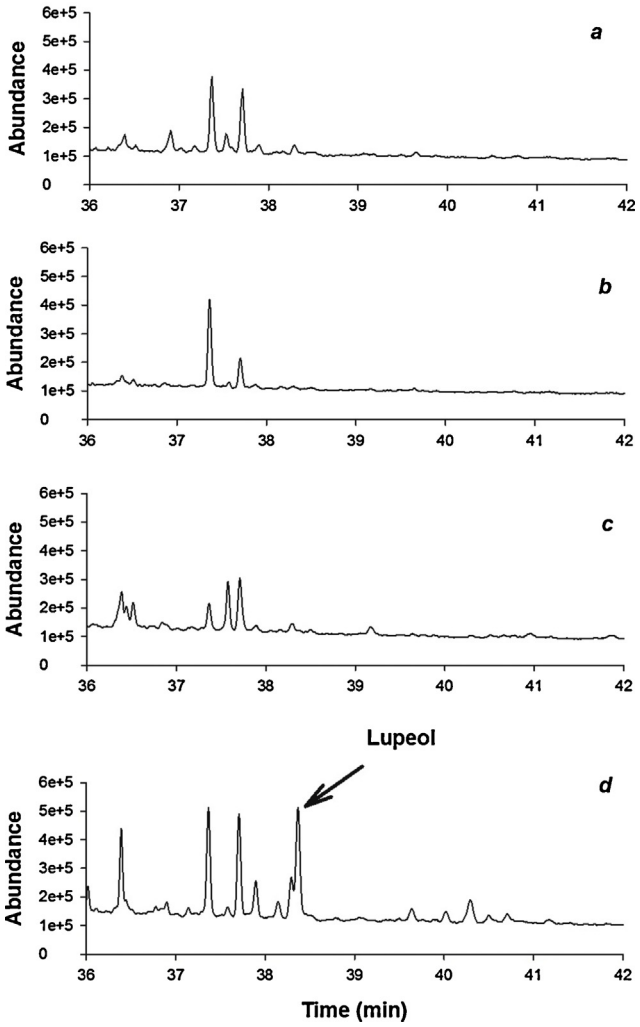

Fig. 1. GC–MS chromatogram of lupeol in white oak species, *Quercus resinosa* (a), *Q. grisea* (b), *Q. laeta* (c) and *Q. obtusata* (d).

obtain lupeol from several white oak species. In addition, a second objective was to establish a method for its efficient extraction and quantification.

2. Materials and methods

2.1. Materials and chemicals

Fresh leaves of white oak species such as *Quercus resinosa*, *Quercus grisea*, *Quercus laeta* and *Quercus obtusata*, were collected at Road Charcos-Mezquital km 30, Southeastern Durango, (23° 01′ North latitude, 104° 18′ West longitude, 2700 m altitude). A minimum of ten trees of each species were used for leaves collect. The sampling collect was random. Leaves of each specie were deposited in the herbal collection of (ISIMA-UJED) and identified by botanic Jeffrey Bacon from ISIMA-UJED. All leaves of each species were blended Mexico. COX (ovine) Inhibitor Screening Assay Kit was obtained from Cayman Chemical (Ann Arbor, MI, USA). Hexane and ethyl acetate were purchased from J.T. Baker (Center Valley, PA, USA).

2.2. Determination of lupeol in aqueous extracts of Quercus species by CG–MS

Leaves of *Quercus resinosa*, *Q. grisea*, *Q. laeta* and *Q. obtusata* were air dried under the shadow at room temperature, and powdered using an IKA mill (Wilmington, NC, USA) and sieved through a 0.147 mm mesh. Aqueous extracts were obtained from 1 g of herb sample added to 100 mL of hot water (80 °C), and kept stirring for 10 min. Subsequently, aqueous extracts were clarified by centrifugation and filtered with filter (pore 0.45 μm). Quantification of lupeol in extracts of *Quercus* species, was performed by GC–MS. Briefly, extracts were subjected to liquid–liquid micro-extraction with ethyl acetate. The solvent was evaporated with nitrogen gas and then 50 μL of derivatizing agent N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) was added and stirred for 2 min at room temperature (25 °C). Finally, 1 μL of derivatized sample was injected to a GC–MS system following a modified method of Razborsek et al. (2008).

The GC–MS system consisted of an Agilent GC Series 7890A and an Agilent single quadrupole MS detector (model 5975C, Santa Clara, CA, USA), with electron energy set at 70 eV and the mass range at 50–700 *m/z*. An HP-5MS capillary column (30 × 0.25 mm i.d. × 0.25 μm) and a split/splitless injector were used. The injector was set at 250 °C. The GC was performed in the splitless mode. The initial oven temperature was held at 100 °C for 1 min and raised to 220 °C at 6 °C/min, held for 1.23 min, then raised to 290 °C at 10 °C/min, and raised 310 °C at 40 °C/min, and held for 7.5 min. The flow rate of carrier gas (helium) was maintained at 1 mL/min. The GC–MS control and data processing was performed using ChemStation (Agilent Technologies) software.