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Review

Recent developments on the extraction and application of ursolic acid. A review

Lucía López-Hortas[a,b], Patricia Pérez-Larrán[a], María Jesús González-Muñoz[a,1], Elena Falqué[b], Herminia Domínguez[a,*]

[a] Departamento de Enxeñería Química, Facultad de Ciencias, Universidade de Vigo, As Lagoas s/n, 32004 Ourense, Spain
[b] Departamento de Química Analítica, Facultad de Ciencias, Universidade de Vigo, As Lagoas s/n, 32004 Ourense, Spain

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ABSTRACT

Ursolic acid (UA) is a pentacyclic triterpenoid widely found in herbs, leaves, flowers and fruits; update information on the major natural sources or agro-industrial wastes is presented. Traditional (maceration, Soxhlet and heat reflux) and modern (microwave-, ultrasound-, accelerated solvent- and supercritical fluid) extraction and purification technologies of UA, as well as some patented process, are summarized. The great interest in this bioactive compound is related to the beneficial effects in human health due to antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, immunomodulatory, anti-tumor, chemopreventive, cardioprotective, anti-hyperlipidemic and hypoglycemic activities, and others. UA may augment the resistance of the skin barrier to irritants, prevent dry skin and could be suitable to develop antiaging products. The development of nanocrystals and nanoparticle-based drugs could reduce the side effects of high doses of UA in organisms, and increase its limited solubility and poor bioavailability of UA which limit the potential of this bioactive and the further applications. Commercial patented applications in relation to cosmetical and pharmaceutical uses of UA and its derivatives are surveyed.

1. Introduction

Ursolic acid (UA) (3β-hydroxy-urs-12-en-28-oic-acid) (Fig. 1) is a pentacyclic triterpenoid carboxylic compound ($C_{30}H_{48}O_3$) which may occur in the free acid form or as aglycones for triterpenoid saponins. Its isomer, oleanolic acid (OA) (3β-hydroxy-olea-12-en-28-oic-acid), presents different substitution of the methyl group, but they have similar molecular structures and pharmacological activity (Lim et al., 2007). Oleanolic acid and ursolic acid are ubiquitous triterpenoids in plant kingdom, medicinal herbs, and are integral part of the human diet, once they are found in fruits and medicinal herbs.

In recent years they became the subject of many publications because of their various activities and low toxicity. Many beneficial effects such as antioxidative, antimicrobial, anti-inflammatory, anticancer, anti-hyperlipidemic, analgesic, hepatoprotectory, gastroprotective, anti-ulcer, anti-HIV, cardiovascular, antiatherosclerotic and immunomodulatory effects have been reported (Kashyap, Tuli, & Sharma, 2016; Liu, 1995; Woźniak, Skąpska, & Marszałek, 2015). Several works have shown that the ursolic acid can also stimulate muscle growth, reduce fat gain and enhance the epidermal permeability barrier recovery in the skin (Kunkel et al., 2012), therefore it has been proposed as a skin therapeutic agent and it could be introduced in sport supplements (Close, Hamilton, Philp, Burke, & Morton, 2016; Deane et al., 2017), cosmetics (De Almeida et al., 2014) and health products (Navina, Lee, & Kim, 2017). During the last decade articles published reported on the isolation and purification of these triterpenoids from various fruits, plants and herbs, the chemical modifications to prepare more effective and water soluble derivatives, the pharmacological research on their beneficial effects, the toxicity studies, and the clinical use of these triterpenoids in various diseases including anticancer chemotherapies (Chen, Gao, et al., 2015; Jin et al., 2016; Liu, 2005; Sultana, 2011). The effects of pentacyclic triterpenes on proinflammatory mediator signaling pathways and data from experimental animal models and clinical trials (Safayhi & Sailer, 1997) and the antitumor and chemopreventive activity and its main anti-tumor effects and chemopreventive properties have been reviewed (Novotny, Vachalkova, & Biggs, 2001; Ovesna, Vachalkova, Horvathova, & Tothova, 2004; Woźniak et al., 2015). More recently, the therapeutic effects of UA, both in prevention and treatment of health disorders, and its mechanisms of action were compiled (Kashyap et al., 2016).

The present review aims at presenting the chemical and biological

* Corresponding author.
E-mail address: herminia@uvigo.es (H. Domínguez).
[1] Present address: Hifas da Terra SL, Portamuiños, 7, 36154 Bora, Pontevedra, Spain.

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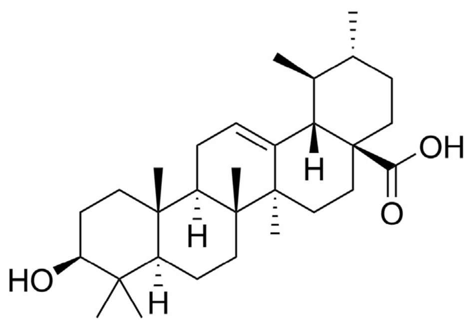

Fig. 1. Chemical structure of ursolic acid.

properties of ursolic acid, their occurrence and natural sources, the conventional and novel extraction technologies and the commercial and patented products formulated with this compound.

2. Sources

Ursolic acid can be isolated from various medicinal plants, *Lamiaceae* family being one of the most known source of triterpenes (Razborsek, Voncina, Dolecek, & Voncina, 2008; Wójciak-Kosior, Sowaa, Kocjan, & Nowak, 2013), with contents up to 2.95% d.w. (Jäger, Trojan, Kopp, Laszczyk, & Scheffler, 2009; Razborsek et al., 2008) in *Rosmarinus officinalis* leaves, one of the traditional commercial sources (Silva et al., 2008). UA has also been identified in a variety of sources, particularly in leaves and flowers (Han et al., 2014; Kowalski, 2007; Takada, Nakane, Masuda, & Ishii, 2010; Xing, Bi, Zhao, & Xia, 2013). The triterpenic acids oleanolic and ursolic were recently detected for the first time in wild edible mushrooms (Kalogeropoulos, Yanni, Koutrotsios, & Aloupi, 2013) and in some commercial dried fruits (Zhang, Daimaru, Ohnishi, Kinoshita, & Tokuji, 2013). Other sources are indicated in Table 1. Cuticular waxes are excellent sources of triterpenoids with a role in protection against biotic stresses, such as herbivores and pathogens, on the mechanical properties of the fruit surface, and these compounds are partially responsible for allelopathic potential (Szakiel & Mroczek, 2007). Triterpene distribution within various plant materials (Jäger et al., 2009) and the triterpenoid profile of cuticular waxes of some edible fruits is compiled (Szakiel & Mroczek, 2007; Szakiel, Pączkowski, Pensec, & Bertsch, 2012). Agro-industrial wastes are advantageous alternative sources. Ursolic acid is the major triterpene in the leaf and fruit of argan, this latter as a by-product of the argan oil industry (Guinda, Rada, Delgado, & Castellano, 2011), and is found in wastes such as those from juice production (Popov et al., 2011; Sorokina et al., 2010), apple peels (Fan et al., 2013; He & Liu, 2007; Lv, Tahir, & Olsson, 2016; Yamaguchi et al., 2008), the discarded by-products from apple or persimmon processing, especially peels (Cargnin & Gnoatto, 2017; Chun, Park, Choung, Kim, & Lee, 2014), unripe and overripe fruits produced on harvesting, i.e. elderberries (Salvador, Rocha, & Silvestre, 2015), and raffinates, such as rosemary leftovers obtained after the extraction of carnosic acid (Liang et al., 2015). Forestry wastes could be another source, i.e. the barks of *Eucalyptus* sp. (de Melo, Oliveira, Silvestre, & Silva, 2012; Domingues et al., 2013; Patinha et al., 2013).

The developmental stage and the environmental conditions modulate the UA biosynthesis (Guinda et al., 2011) and seasonal variations were observed during ripening in fruits and leaves of olive tree cultivars (Peragón, 2013), in *Silphium* sp. (Kowalski, 2007), in apple peels (Lv et al., 2016), in *S. integrifolium* and *S. trifoliatum* leaves before and at the beginning of flowering (Kowalski, 2007). The levels of sugars and most triterpenic acids increased with ripening in jujube (*Ziziphus jujuba*) fruit (Guo et al., 2015). Ursolic and oleanolic acids, the most abundant compounds in lipophilic extractives of *Sambucus nigra* L., followed by smaller amounts of long chain aliphatic alcohols and sterols, showed an initial growth during ripening and a systematic decrease until maturity (Salvador et al., 2015). The relative levels of triterpenoid individual compounds can vary in consecutive years due to the influence of external abiotic and biotic stimuli (e.g., meteorological conditions, pathogen infections). A considerable decrease in the level of oleanolic acid was observed during fruit development, probably due to an increase in the level of aliphatic constituents of cuticular waxes. During fruit development, the total triterpenoid content decreased, and in mature grapes, the total triterpenoid content ranged from 34 to 49% of the wax extract mass (Pensec et al., 2014). The variation of triterpenoids in fruits and leaves of olive tree cultivars during processing was also reported (Peragón, 2013).

Oleanolic acid, oleanolic and ursolic acid methyl esters, and oleanolic aldehyde are among the common compounds identified in eight grape cultivars grown in the Upper Rhine Valley. The total triterpenoid content significantly differed among cultivars, ranging from 42 to 80% (Pensec et al., 2014).

The UA content varied in different parts of the plant and this pattern of distribution may have an important physiological and ecological meaning (Szakiel & Mroczek, 2007). Higher contents in the inflorescences and leaves than in roots were observed (Kowalski, 2007), in the leaf of argania were four times higher than in the fruit (Guinda et al., 2011). Free triterpene acids and small amounts of the oleanolic and UAs bound forms (presumable glycosides and glycoside esters) occur in fruits and the vegetative part of *Vaccinium vitis-idaea* L. (Szakiel & Mroczek, 2007). The total content of both acids was the highest in fruits and leaves, lower in stems and rhizomes, these latter contained more bound forms of both acids.

UA content in the samples from different sources were significantly different (Yang, Wei, Chiu, & Huang, 2013), and the geographic variation was also observed in *Paulownia* fortune (Li et al., 2011), *Ocimum* species (Silva et al., 2008), *Argania spinosa* (Guinda et al., 2011). The outer barks of *Eucalyptus* trees from temperate and Mediterranean zones are richer in triterpenic acids than the species from sub-tropical and tropical regions (Domingues et al., 2011).

3. Extraction

Table 1 summarizes the extraction yields from different sources and techniques. Some patented processes are summarized in Table 2.

The key aspects affecting the extraction process include the operational variables influencing the yields and rates, such as the type of solvent and solvent to solid ratio, extraction time and temperature, but also mechanical and thermal sample conditioning before extraction is an essential step that should be optimized. Mainly researches were carried out at laboratory-scale. The influence of the material preparation on the ursolic acid extraction performance from *Paulownia fortunei* leaves was confirmed by a reduction in the time required to reach maximum extraction from 20 min with conventional powder to 5 min with ultramicro powder. In addition, the extraction of UA from ultramicro powder was nearly twice that reached with conventional powder (Yu, Zhao, Shao, Chen, & Chen, 2008). The effect of drying and cleaning has also been reported (Fernández-Hernández, Martínez, Rivas, García-Mesa, & Parra, 2015; Junhai, Hongguang, Zhizhou, & Feng, 2012; Lee, Park, Pyo, & Kim, 2010).

Traditional extraction techniques, such as maceration, Soxhlet extraction and heat reflux extraction, have been reported mainly for the extraction of ursolic acid from fruits and medicinal, and depending of the raw material, the method and/or solvents were chosen. Conventional solvents such as water, lower alcohols and their mixtures with water, acetone, diethylether, chloroform, hexane, and ethyl acetate have been compared for the extraction of ursolic acid from seeds (Kim & Kim, 2000) and fruits (Xia et al., 2012). Absolute ethanol has been proposed and selected for the extraction of pentacyclic triterpenic acids from *A. spinosa* (Guinda et al., 2011), ursolic and oleanolic acids from *Ligustrum lucidum* (Futing et al., 2012), ursolic acid from leaves of *Paulownia* (Xu, Sheng, Yan, & Li, 2012), perilla leaves