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Artículo de Investigación

URSOLIC ACID: AN OVERVIEW INCLUDING RESEARCH PERFORMED IN PERU

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ABSTRACT

Ursolic acid (3β-3-hydroxy-urs-12-en-28-oic-acid) is a pentacyclic triterpenoid compound present in many medicinal herbs and edible fruits of different species of plants. Ursolic acid is now considered an important biomolecule due to its pharmacological activity and much of the research has focused on anticancer activity. To achieve the clinical application of ursolic acid, delivery nanosystems have been developed and the synthesis of its derivatives has also been carried out. In this review, we address different aspects of the chemistry of ursolic acid. Furthermore, we highlight the investigations that were carried out in Peru concerning ursolic acid.

Keywords: Ursolic acid, Triterpenoid, Lamiaceae, Medicinal plants.

ÁCIDO URSÓLICO: UNA REVISIÓN GENERAL QUE INCLUYE INVESTIGACIONES REALIZADAS EN PERÚ

RESUMEN

El ácido ursólico (ácido 3β-hidroxi-urs-12-en-28-óico) es un triterpenoide pentacíclico presente en varias hierbas medicinales y frutos comestibles de diferentes especies de plantas, es considerado una importante biomolécula debido a su actividad farmacológica y gran parte de las investigaciones se han enfocado en su actividad anticancerígena. Para lograr la aplicación clínica del ácido ursólico, se han desarrollado nanosistemas para su administración y también se han sintetizado gran cantidad de derivados. En esta revisión abordamos diferentes aspectos de la química del ácido ursólico, y además, destacamos las investigaciones que se llevaron a cabo en el Perú.

Palabras clave: Ácido ursólico, Triterpenoide, Lamiaceae, Plantas medicinales.

ÁCIDO URSÓLICO: UMA REVISÃO GERAL INCLUINDO PESQUISAS REALIZADAS NO PERÚ

RESUMO

O ácido ursólico (ácido 3β-hidroxi-urs-12-en-28-óico) é um triterpenóide pentacíclico presente em muitas ervas medicinais e frutas comestíveis de diferentes espécies de plantas, o ácido ursólico é considerado uma biomolécula importante devido à sua atividade farmacológica e muitas pesquisas têm focalizado sua atividade anticancerígena. Para alcançar a aplicação clínica do ácido ursólico, desenvolveram-se nanosistemas para sua administração e também se realizou a síntese de seus derivados. Nesta revisão, abordamos diferentes aspectos da química do ácido ursólico e, além disso, destacamos as pesquisas realizadas no Perú.

Palavras-chave: Ácido ursólico, Triterpenóide, Lamiaceae, Plantas medicinais.

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INTRODUCTION

The triterpenoids are a large group of natural compounds biosynthesized through the cyclization of 2,3-oxidosqualene (Cárdenas et al., 2019; Yao et al., 2020) and among more than 20,000 different structures known so far, the ursolic acid (UA) (3β-3-hydroxy-urs-12-ene-28-oic-acid) is widely distributed in different vegetal species, including the cuticular wax from fruits edible and medicinal herbs (Jäger et al., 2009; Szakiel et al., 2012; López et al., 2018). Several analytical methods have been developed for qualitative and quantitative analysis of UA in different raw materials. Chromatographic analytical methods such as HPLC, UPLC, and GC are the most employed for the analysis of UA (Pironi et al., 2018). However, in recent years electrochemical methods have also been developed (Feng et al., 2020). UA and their synthetic derivatives displays different pharmacological activities such as anticancer (Kashyap et al., 2016; Khwaza et al., 2020), antidiabetic (Numonov et al., 2020), anti-inflammatory (Wei et al., 2018), antioxidant (Habtemariam, 2019), and antimicrobial (Jyoti et al., 2016). The biological activity of UA is attributed to its chemical structure, especially the hydroxyl and carboxyl group at the C-3 and C-28 positions, respectively (Vo et al., 2019). These groups facilitate chemical modification to improve the bioactive properties of UA and to develop new bioactive agents (Zhou et al., 2017). Furthermore, due to the lipophilic character of UA, it can interact with phospholipids and cause a disruption of the integrity of the membrane, which allows it to enter and influence the activity of various enzymes (Fajardo et al., 2017). Nevertheless, the low solubility in water and poor bioavailability of UA hinders its preclinical or clinical application. Currently, various strategies are developed to improve or overcome these disadvantages, for example, chemical modification of the UA skeleton (Hodon et al., 2019), and nanosystems for the delivery of UA (Shao et al., 2020). Notable progress has been made in the formulation of UA-nanoliposomes in subjects with advanced solid tumors (Qian et al., 2015). In this review, we address all these points in a general way and also highlight the investigations that were carried out in Peru concerning UA.

1. Structure of ursolic acid

Ursolic acid or (3β-3-hydroxy-urs-12-ene-28-oic-acid) is an ursane-type pentacyclic triterpenoid, which has the chemical formula of $C_{30}H_{48}O_3$ and a molecular mass of 456.7 g/mol, with a melting point of 283–285 °C (Chan et al., 2019). It has a complex structure with a skeleton of 30-carbons of which ten are chiral centers (**Figure 1**). UA is poorly soluble in water, but it is easily dissolved in ethanol (Fan et al., 2011). The crystal structure of UA is orthorhombic with $P2_12_12_1$ space group, a(Å) = 7.199 (1), b(Å) = 12.157 (2), c(Å) = 33.888 (2), and formula unit per cell (Z = 4), ethanol solvate (Zhou et al., 2015). The characteristic spectroscopic signals of UA are as follows: The UV spectrum shows maximum absorption wavelength of ~450 nm. The IR spectrum shows strong

absorptions at 3562 cm^{-1} (OH alcohol), 2937 cm^{-1} (OH acid), 2865 cm^{-1} (C=C) and 1698 cm^{-1} (C=O). The ^{13}C-NMR spectrum shows 30 carbon signals, of which seven quaternary carbons, seven methines, nine methylenes and seven methyls are deduced from the DEPT experiments (Babalola & Shode, 2013). The chemical shift at δ 180.0 is attributed to the carboxylic acid (C-28), δ 78.1 to the hydroxyl (C-3), δ 125.6 and δ 139.7 to double bond C-12 and C-13 respectively (Uddin et al., 2011). Integrating the information of 1D-NMR (^{1}H, ^{13}C and DEPT) with 2D-NMR (COSY, HSQC and HMBC) the signals corresponding to each carbon and hydrogen can be identified (Ludeña & Tupa, 2017).

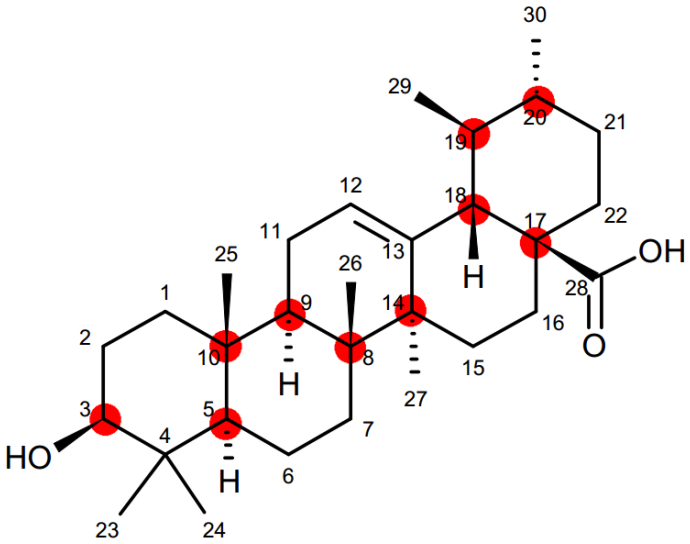

Figure 1. Structure of ursolic acid (UA) and chiral centers (●) (Adapted from SciFinder 77-52-1)

2. Sources of ursolic acid

UA represents a family of pentacyclic triterpenes and is widely distributed in different species of plants. In the literature, there are many phytochemical studies on the identification and quantification of UA in numerous medicinal herbs, including *Plantago major* (Kartini et al., 2014), *Ocimum sanctum* (Vetal et al., 2014), *Psychotria viridis* (Soares et al., 2017), *Sinningia mauroana* (Winiewski et al., 2020), and other species. In particular, plants belonging to the Lamiaceae family are characterized by containing UA in the various organs of the plant, which can be considered as a taxonomic marker (Janicsák et al., 2006; Silva et al., 2008). On the other hand, the cuticular wax responsible for the wettability and permeability properties of the cuticle, is also a source of biologically active pentacyclic triterpenoids (Szakiel et al., 2012). UA has been identified in the cuticular wax of many edible fruits, such as cherry, loquat, pear, peach, quince, apple, bilberry and high amounts of this triterpene had been detected in the cuticular wax of five fruits of the Rosaceae family (Ludeña & Ramos, 2019) (**Figure 2**). Regarding the preparation of UA, to the best of our knowledge, only two works have developed an easy and economically feasible method to obtain

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UA crystals, both works use the recrystallization method. However, Fan et al. (2016) uses the apple peel as raw material and Ludeña (2018) uses the medicinal herb *Clinopodium revolutum* belonging to the Lamiaceae family as raw material.

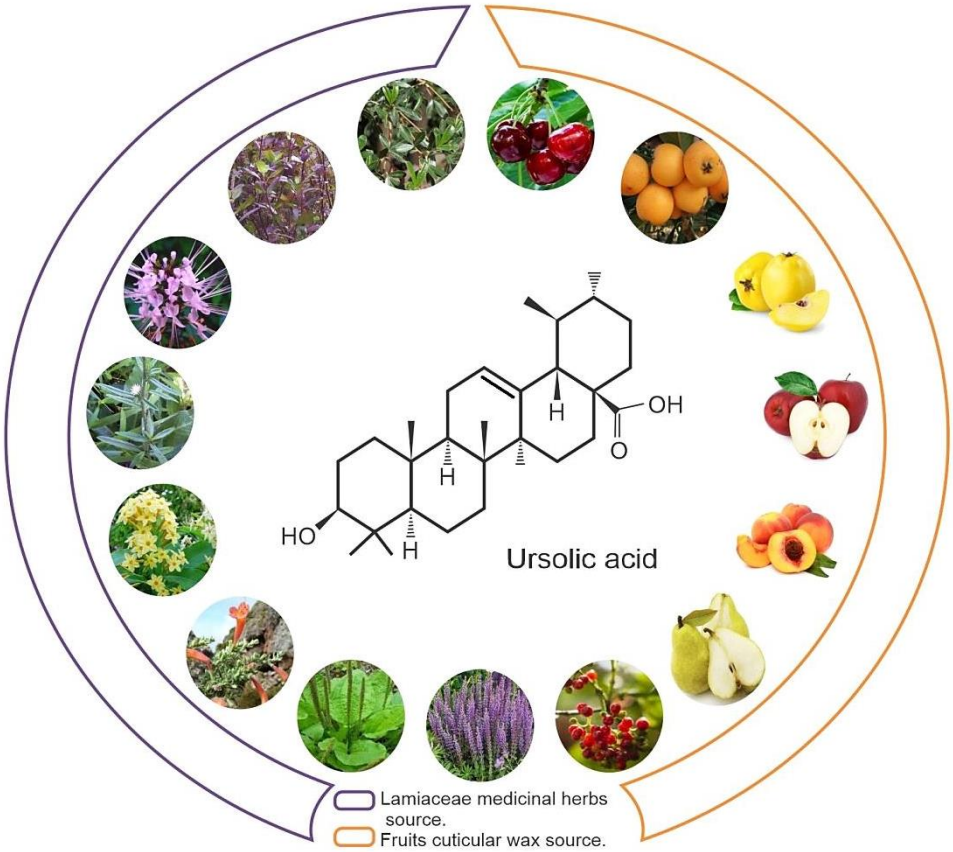

Figure 2. Medicinal herbs and cuticular wax from different fruits in which ursolic acid has been identified, quantified or extracted (own elaboration)

3. Biosynthesis of ursolic acid

Pentacyclic triterpenoids form a large family of complex chemical structures. These compounds are biosynthesized in the cytosol via the mevalonate pathway (Adam et al., 1999) (Chappell, 2002). Briefly, the mevalonate (MVA) pathway starts with molecules of acetyl-CoA and involves a series of enzymes that promote their condensation to give rise to the precursors identified as isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (Miziorko, 2011). Different prenyltransferases catalyze the sequential chain elongation of DMAPP with IPP to synthesize the intermediate farnesyl pyrophosphate (FPP), which is converted to squalene by squalene synthase (SQS) and, then squalene is oxidized to 2,3-oxidosqualene by squalene epoxidase (SQE) (Phillips et al., 2006; Thimmappa et al., 2014). The cyclization of 2,3-oxidosqualene by oxidosqualene cyclases (OSCs) is the first step that gives rise to the biosynthesis of sterol or triterpene scaffolds, the chain-chain-chain conformation organizes cyclization to generate simple triterpene scaffolds

such as α-amyrin, β-amyrin, and lupeol (Xu et al., 2004; Abe, 2007). These triterpene scaffolds are modified by cytochrome P450 monooxygenases, leading to the corresponding C-28 oxidized products (Seki et al., 2015; Ghosh, 2017). In particular, the α-amyrin is a precursor of UA, which is generated by cyclization of 2,3-oxidosqualene by the OSC α-amyrin synthase (aAS). Subsequently, the CYP716A enzyme modifies this triterpenol, which catalyzes three-step oxidation at the C-28 position of α-amyrin to produce UA through uvaol and ursolic aldehyde (**Figure 3**) (Lu et al., 2018; Suzuki et al., 2019; Srisawat et al., 2019).

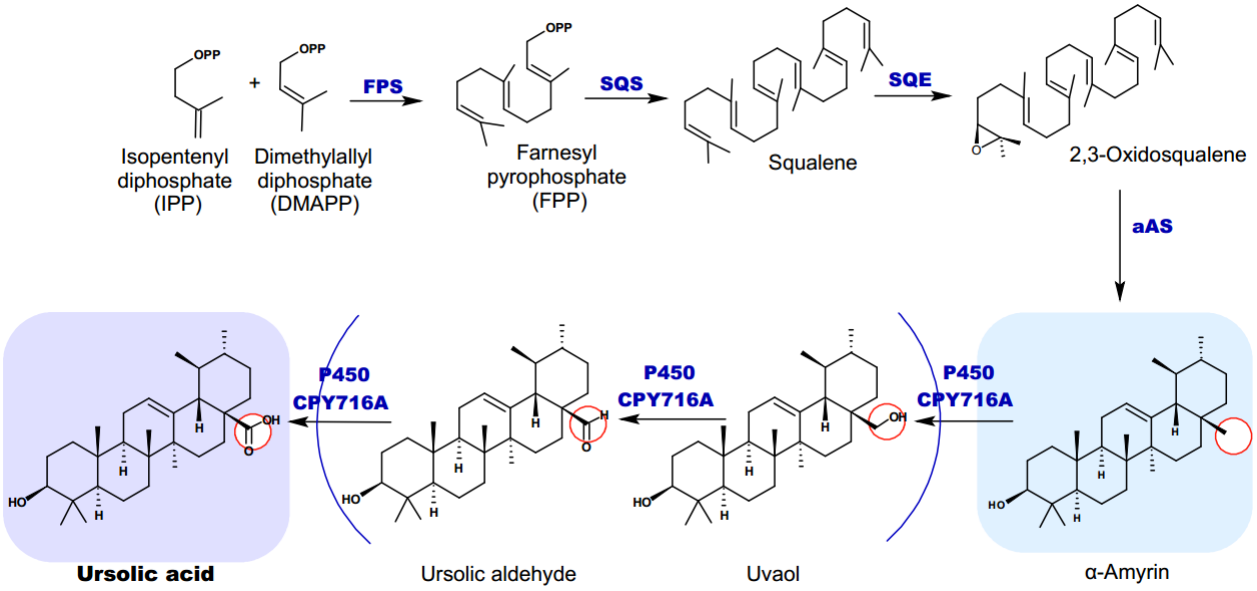

Figure 3. Scheme of the biosynthesis of ursolic acid. The enzymes that catalyze the different steps are: FPS, farnesyl pyrophosphate synthase; SQS, squalene synthase; SQE, squalene epoxidase; aAS, α-amyrin synthase. Modification of the triterpene α-amyrin in three-step oxidations catalyzed by CYP716A enzymes at position C-28 (own elaboration)

4. Detection of ursolic acid

Up to now, several analytical methods for the determination of UA in different matrices have been developed and validated. Among these, TLC is excellent for the preliminary chemical evaluation in plant extracts (Jamal et al., 2018). Furthermore, TLC allows the separation and identification of pentacyclic triterpenes of similar structure such as oleanolic acid, lupeol, etc (Mučaji & Nagy, 2011; Martelanc et al., 2016). However, HPLC is the preferred technique due to its simplicity, excellent sensitivity, and resolution (Li et al., 2019). GC is also used for the separation, quantification and structural determination of UA, the advantage of this technique is its sensitivity and precision in the analysis (Razboršek et al., 2008). Nonetheless, it is mandatory to perform derivatization (Silylation or acetylation) to all pentacyclic triterpenoids prior to their GC-FID or GC-MS analysis (Jemmali et al., 2016). Spectroscopic methods such as one-dimensional (1D) and two-dimensional (2D) NMR spectroscopy can also be used for the identification and quantification

of various pentacyclic triterpenoids, including UA, in complex matrices of plant extracts (Kontogianni et al., 2009; Palu et al., 2019). Lacikova et al. (2006) developed and validated a direct and specific method by tandem mass spectrometric (MS-MS) for the quantification of UA in the leaves of different plant species, without the need for chemical derivatization or chromatographic separation of the herbal matrix. Capillary electrophoresis (CE) has also been used, which is an inexpensive and useful technique to investigate pentacyclic triterpenoids with the advantages of remarkable separation efficiency, simplicity, reproducibility, and speed (Yang et al., 2007; Gao et al., 2015). Although most research has been based on the use of chromatographic techniques, currently there are studies in which electrochemical methods are used in the detection and quantification of UA (Tyszczuk et al., 2015). For example, differential pulse voltammetry (DPV) in which the potential changes linearly with time (linear sweep of potential) superimposed by potential pulses of amplitude between 10 and 100 mV, over several milliseconds. This technique has shown high sensitivity, rapid response, and low limit of detection in the quantification of UA (Oancea et al., 2019; Feng et al., 2020).

5. Ursolic acid: a look from inside the Peru

In order to know the investigations carried out in Peru to date, concerning UA, an exhaustive search was carried out in academic databases such as SciFinder, SciELO, PubMed, Dialnet, DOAJ, Biblat, ScienceDirect and search websites such as Google Scholar. Despite the fact that Peru is one of the twelve megadiverse countries on the planet with a large number of plant species with medicinal properties used by the population (Herrera et al., 2019), only seven academic publications upon UA have been found. The Lamiaceae are represented in Peru by around 21 genera and 190 species, of which 57 are recognized as endemic species (Rodriguez, 2013). Further, Peru annually produces tons of Rosaceae edible fruits such as apple, pear, peach and also has different Rosaceae native fruits such as *Hesperomeles heterophylla, Prunus serotina,* and *Rubus glaucus* known as "millucapa", "capulí", and "zarzamora", respectively (Mostacero et al., 2017). From the foregoing, it is clear that there are several plants still available in nature to investigate, and not just about UA. The investigations carried out in Peru on UA, consist of the identification (3 articles), quantification (3 articles) and preparation (1 article). Neto et al. (2000) contributed to the identification of four known triterpenoids in the ethanolic extract of *Polylepis racemosa*, known as "queñual", which is used in traditional Peruvian medicine to treat uterine cancer and inflammation, among these triterpenoids, UA was identified by ^{1}H- and ^{13}C-NMR, now the use of this plant to treat uterine inflammation may be explained by the presence of UA, a powerful natural anti-inflammatory compound. Later, Kawano et al. (2009) presented the active components in the methanolic extract of *Cestrum auriculatum* and *Cestrum hediundinum,* both medicinal herbs are known as ''Hierba

santa" and are used to alleviate many kind of symptoms, including headache, hemorrhoids, fever, and rheumatism, UA was identified in the extract of *Cestrum auriculatum* by direct comparison of ^{1}H-NMR and TLC with the authentic compound. The traditional remedy *Pseudelephantopus spicatus*, known as "mata pasto" used by the Chayahuita, an ethnic group from the Peruvian Amazonia, was studied by Odonne et al. (2011). They discovered in the ethanolic extract the active compounds of this plant, among these compounds UA was identified by ^{1}H and ^{13}C-NMR. In the last year, Serrano et al. (2020) as part of their research project on the evaluation of pentacyclic triterpenoids in Peruvian species of Lamiaceae, identified and quantified by HPLC the content of UA and other triterpenoids in thirteen Peruvian species of Lamiaceae (**Table 1**) (Serrano et al., 2016).

Table 1. List of Peruvian plants in which ursolic acid has been identified or quantified. Information is included on the sites where these plants were collected, the organ that was analyzed and the method used (own elaboration)

Species	Plant origin	Amount of AU; Plant part; Method
Lepechinia meyenii (Lamiaceae)[a]	Cusco	7.9 mg/g; Dried aerial; HPLC
Clinopodium brevicalyx (Lamiaceae)[a]	Cusco	7.7 mg/g; Dried aerial; HPLC
Salvia oppositiflora (Lamiaceae)[a]	Cusco	5.8 mg/g; Dried aerial; HPLC
Lepechinia floribunda (Lamiaceae)[a]	Cusco	5.3 mg/g; Dried aerial; HPLC
Minthostachys mollis (Lamiaceae)[a]	Lima	17.6 mg/g; Dried aerial; HPLC
Salvia sagittata (Lamiaceae)[a]	Huánuco	19.7 mg/g; Dried aerial; HPLC
Salvia cuspidate (Lamiaceae)[a]	Lima	14.2 mg/g; Dried aerial; HPLC
Clinopodium revolutum (Lamiaceae)[a]	Huánuco	48.1 mg/g; Dried aerial; HPLC
Clinopodium sericeum (Lamiaceae)[a]	Amazonas	33.4 mg/g; Dried aerial; HPLC
Salvia haenkei (Lamiaceae)[a]	Moquegua	19.5 mg/g; Dried aerial; HPLC
Salvia dombeyi (Lamiaceae)[a]	Lima	11.8 mg/g; Dried aerial; HPLC
Hedeoma mandoniana (Lamiaceae)[a]	Cusco	13.3 mg/g; Dried aerial; HPLC
Clinopodium pulchellum (Lamiaceae)[a]	Ancash	15.8 mg/g; Dried aerial; HPLC
Clinopodium bolivianum (Lamiaceae)[b]	Puno	4.7 mg/g; Dried aerial; HPLC
Polylelis racemosa (Rosaceae)[c]	Ancash	Identified; Bark and stem; ^{1}H and ^{13}C-NMR
Pseudelephantopus spicatustus (Asteraceae)[d]	Amazonas	Identified; Leaves; ^{1}H and ^{13}C-NMR
Cestrum auriculatum (Solanaceae)[e]	Cusco	Identified; Dried aerial; ^{1}H-NMR and TLC
Cestrum hediundinum (Solanaceae)[e]	Amazonas	No present
Malus domestica (Rosaceae)[f]	Cusco-market	9.38 mg/g; Dried peel; HPLC
Prunus pérsica (Rosaceae)[f]	Cusco-market	2.97 mg/g; Dried peel; HPLC
Pyrus communis (Rosaceae)[f]	Cusco-market	7.25 mg/g; Dried peel; HPLC
Chaenomeles japônica (Rosaceae)[f]	Cusco-market	5.69 mg/g; Dried peel; HPLC
Eriobotrya japonica (Rosaceae)[f]	Cusco-market	8.01 mg/g; Dried peel; HPLC

[a]Serrano et al. (2020), [b]Serrano et al. (2016), [c]Neto et al. (2000), [d]Odonne et al. (2011), [e]Kawano et al. (2009), [f]Ludeña & Ramos, 2019.

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Also, they evaluated in these species the content of total phenols, flavonoids, hydroxycinnamic acids, and antioxidant capacity. Other publication examines the content of UA but in the cuticle wax of different edible fruits (quince, loquat, pear, peach and apple) all belonging to the Rosaceae family, the data obtained exhibited that the UA is in greater quantities (**Table 1**) (Ludeña & Ramos, 2019). Ludeña (2018) worked on a preparative method to obtain UA from the endemic Peruvian species *Clinopodium revolutum* known as "flor de arena". The technique used was selective recrystallization, and the process consists of the extraction with ethyl acetate from the leaves, then crystallization and recrystallization with ethanol (96% v/v) were carried out. With this method, he obtained crystals of UA with purity greater than 95%, without the need for preparative chromatographic methods with silica gel or the use of toxic solvents.

CONCLUSIONS AND FUTURE PERSPECTIVES

The evidence highlighted in this review revealed that *Clinopodium revolutum* (Lamiaceae) and *Malus domestica* (Rosaceae) are the species that accumulate the highest amount of UA. These two species can be considered the main sources of UA, which has been proven to have great pharmacological potential. Numerous studies have been conducted on UA, for example, the synthesis of its derivatives and delivery nanosystems with better pharmacokinetic properties, with which it has been possible to overcome the problem of solubility and bioavailability of UA. In addition, the biosynthesis of UA has been elucidated and several investigations have led to the identification and characterization of many cytochromes P450 that are responsible for the biosynthesis of triterpenoids. The UA has a very complex structure with ten chiral centers, for this reason it has not yet been possible to carry out the synthesis in the laboratory and to increase its production the bioengineering procedures could be a successful approach. The researches carried out in Peru are negligible compared to what has been done globally to date. However, the *Clinopodium revolutum* is an endemic species of Peru that could be an alternative for traditional crops such as potatoes, corn, beans, etc. This would generate better economic income for farmers because UA is one of the most expensive biomolecules, with an approximate cost of 280 USD for 10 mg of UA on the Sigma-Aldrich website.

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