

# The Pentacyclic Triterpenes $\alpha$ , $\beta$ -amyryns: A Review of Sources and Biological Activities

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## 1. Introduction

Pentacyclic triterpenes are ubiquitously distributed throughout the plant kingdom, in a free form as aglycones or in combined forms, and have long been known to have a number of biological effects. The compounds  $\alpha$ -amyryn and  $\beta$ -amyryn are commonly found in medicinal plants and oleo-resin obtained by bark incision of several species of *Bursera* or *Protium* of the Burseraceae family. Both *in vitro* and *in vivo* studies have shown that  $\beta$ -amyryn also has important biological functions.

In light of the considerable interest recently generated in the chemistry and pharmacological properties of amyryns and their analogs, we have undertaken this review in an effort to summarize the available literature on these promising bioactive natural products. The review will detail the recent studies on the chemistry and bioactivity of  $\alpha$ ,  $\beta$ -amyryns, which is presented in the following sections: the isolation and distribution of  $\alpha$ -amyryn and  $\beta$ -amyryn, giving a brief introduction to amyryns as natural products and the methods used in their isolation; the biological activities of amyryns, examining the biological properties associated with these compounds with a focus on their potential chemotherapeutic applications.

## 2.1 Chemical structure, detection, analysis and sources

### 2.1.1 Structure

The chemical structure of  $\alpha$ -amyryn (3 $\beta$ -hydroxy-urs-12-en-3-ol) is shown in Fig. 1. The chemical formula of  $\alpha$ -amyryn is C<sub>30</sub>H<sub>50</sub>O, its melting point is 184-186 °C (Sirat, et al., 2010), and it presents an MS ion Peak at m/z 426 (M<sup>+</sup>) (Dias et al., 2011). The infra-red spectrum of  $\alpha$ -amyryn is IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 3450, 2895 and 2895. The chemical structure of  $\beta$ -amyryn (3 $\beta$ -hydroxy-olean-12-en-3-ol) is also depicted in (Fig. 1) and its formula is C<sub>30</sub>H<sub>50</sub>O. The infra-red spectrum of  $\beta$ -amyryn shows the presence of a hydroxyl function and the olefinic moiety at a spectrum of 3360 and 1650 cm<sup>-1</sup> and MS studies of  $\beta$ -amyryn confirm a parent ion peak at m/z 426 (M<sup>+</sup>) (Dias et al., 2011), other work of HR-EI-MS m/z: 426.2975 (calcd. for C<sub>30</sub>H<sub>50</sub>O, 426.3861) (Jabeen et al., 2011). The melting point of  $\beta$ -amyryn is 189-191 °C (Lin et al., 2011).

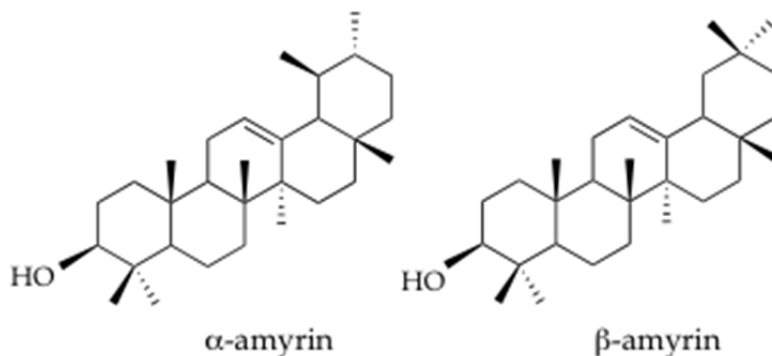


Fig. 1. Estructure of amyrins

NMR methods have indisputably become the single most important spectroscopic techniques for the identification and structure elucidation of amyrins. Several 1D and 2D NMR methods are now commonly used for the characterization of pentacyclic triterpenes. These methods include  $^1\text{H}$  and  $^{13}\text{C}$ -NMR, APT, DEPT, COSY, HMQC, HMBC and TOCSY. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR assignments of  $\alpha$ -  $\beta$ -amyrin are presented in Table 1, (Dias et al., 2011).

### 2.1.2 Detection

Amyrins are found in various plants and plant materials such as leaves, bark, wood, and resins. This material has to be pre-treated prior to isolation of the target compounds. First, the plant material is usually dried, then ground into a powder and sieved. Second, extractions are carried out with dichloromethane, chloroform, *n*-hexane, and methanol. The samples can be subjected to alkaline hydrolysis, derivatization and separation by thin layer chromatography, and the resulting material can be directly subjected to analysis. Gas chromatography (CG) and high performance thin layer chromatography (HPTLC) techniques are the most commonly employed methods to quantitate  $\alpha$ -,  $\beta$ -amyrin in plants.

TLC provided an easy and rapid way to study plant extract profiles and partially identify compounds. The first step for the identification of  $\alpha$ -amyrin,  $\beta$ -amyrin and 3-*epi*-lupeol was to compare  $R_F$  values of reference standards with those of sample extracts. TLC on silica gel revealed that  $\alpha$ -amyrin on tracks 6 and 15,  $\beta$ -amyrin on track 14 and the  $\alpha$ -,  $\beta$ -amyrin mixture on track 16, as well as two standards, all had the same  $R_F$  (Fig. 2). The  $\alpha$ -amyrin band was observed as brown, while the  $\beta$ -amyrin band appeared violet, as did the band for the  $\alpha$ -,  $\beta$ -amyrin mixture. TLC analysis revealed the presence of  $\alpha$ -amyrin,  $\beta$ -amyrin and 3-*epi*-lupeol by a comparison of the position and color of the triterpene spots with those of authentic compounds (Fig. 2). The bands of  $\alpha$ - and  $\beta$ -amyrin or their mixture were observed in all commercial resin tracks 1-5 and medicinal plant tracks 8-13, while 3-*epi*-lupeol track 7 was detected only in the commercial Mexican Copal resins tracks 1-4. Attempts were made to separate the  $\alpha$ -,  $\beta$ -amyrin mixture, which had appeared homogenous on TLC, but without success. These results showed that TLC can be used as a simple method for a preliminary analysis of these triterpenes in extracts of commercial resins and plants, but cannot be employed for the analysis of the  $\alpha$ -,  $\beta$ -amyrin mixture. (Hernández-Vázquez et al., 2010)

| Position | $\alpha$ -amyrin                                    |                       | $\beta$ -amyrin                                     |                       |
|----------|---|-----------------------|---|-----------------------|
|          | $\delta^1\text{H}$                                  | $\delta^{13}\text{C}$ | $\delta^1\text{H}$                                  | $\delta^{13}\text{C}$ |
| 1        |   | 38.7                  |   | 38.7                  |
| 2        |   | 28.7                  |   | 27.2                  |
| 3        | 3.16 ( <i>dd</i> , $J = 5.1; 11.2$ )                | 79.6                  | 3.15 ( <i>dd</i> , $J = 4.4; 10.8$ )                | 79.3                  |
| 4        |   | 38.7                  |   | 38.5                  |
| 5        | 0.67 ( <i>d</i> , $J = 11.6$ )                      | 55.1                  | 0.68 ( <i>d</i> , $J = 11.0$ )                      | 55.1                  |
| 6        |   | 18.4                  |   | 18.6                  |
| 7        |   | 32.2                  |   | 32.4                  |
| 8        |   | 40.7                  |   | 39.8                  |
| 9        |   | 47.7                  |   | 47.6                  |
| 10       |   | 36.6                  |   | 36.9                  |
| 11       |   | 23.3                  |   | 23.6                  |
| 12       | 5.06 ( <i>t</i> , $J = 3.2$ )                       | 124.4                 | 5.12 ( <i>t</i> , $J = 3.2$ )                       | 121.7                 |
| 13       |   | 139.5                 |   | 145.2                 |
| 14       |   | 42.0                  |   | 41.7                  |
| 15       | 1.94 ( <i>td</i> , $J = 4.5; 13.5 \text{ H}\beta$ ) | 27.2                  | 1.89 ( <i>td</i> , $J = 4.0; 14.0 \text{ H}\beta$ ) | 26.2                  |
| 16       | 1.76 ( <i>td</i> , $J = 5.0; 13.5 \text{ H}\beta$ ) | 26.6                  | 1.70 ( <i>td</i> , $J = 4.3; 13.5 \text{ H}\beta$ ) | 26.1                  |
| 17       |   | 33.7                  |   | 32.6                  |
| 18       |   | 59.0                  |   | 47.2                  |
| 19       |   | 39.6                  | 1.93 ( <i>dd</i> , $J = 4.0; 13.7 \text{ H}\beta$ ) | 46.8                  |
| 20       |   | 39.6                  |   | 31.0                  |
| 21       |   | 31.2                  |   | 34.7                  |
| 22       | 1.85 ( <i>dt</i> , $J = 3.0; 7.0$ )                 | 41.5                  | 1.80 <i>m</i>                                       | 37.1                  |
| 23       | 0.93 <i>s</i>                                       | 28.1                  | 0.77 <i>s</i>                                       | 28.0                  |
| 24       | 0.74 <i>s</i>                                       | 15.6                  | 0.90 <i>s</i>                                       | 15.4                  |
| 25       | 0.73 <i>s</i>                                       | 15.6                  | 0.73 <i>s</i>                                       | 15.4                  |
| 26       | 0.89 <i>s</i>                                       | 16.8                  | 0.93 <i>s</i>                                       | 16.8                  |
| 27       | 1.01 <i>s</i>                                       | 23.2                  | 1.19 <i>s</i>                                       | 25.9                  |
| 28       | 0.94 <i>s</i>                                       | 28.1                  | 1.07 <i>s</i>                                       | 28.4                  |
| 29       | 0.85 ( <i>d</i> , $J = 6.0$ )                       | 17.4                  | 0.87 <i>s</i>                                       | 33.8                  |
| 30       | 0.73 ( <i>d</i> , $J = 7.0$ )                       | 21.4                  | 0.80 <i>s</i>                                       | 23.7                  |

Table 1. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR Spectral Data of  $\alpha$ - and  $\beta$ -amyrin

$\alpha$ -Amyrin,  $\beta$ -amyrin and other triterpenes were analysed by TLC and HPLC, the chromatographic techniques including silica gel and reversed-phase (C18RP) TLC and C18 RP-HPLC using UV and mass spectrometric (MS) detection with APCI (Martelanc et al., 2009). HPTLC combined with densitometry has been used to analyse the triterpenoids  $\alpha$ -,  $\beta$ -amyrin and the oleanolic acid content of acetone and ethyl acetate extracts of the leaves of *Jovibara sobolifera* (Sims) (Szewczyk et al., 2009). The detection and/or quantitation of  $\alpha$ -,  $\beta$ -amyrin either in plants or plant products using GC methods requires pre-derivatization of the samples, for example, acetylation or trimethylsilylation. Sometimes a sample clean-up employing silica gel columns or liquid-liquid partition is also necessary.

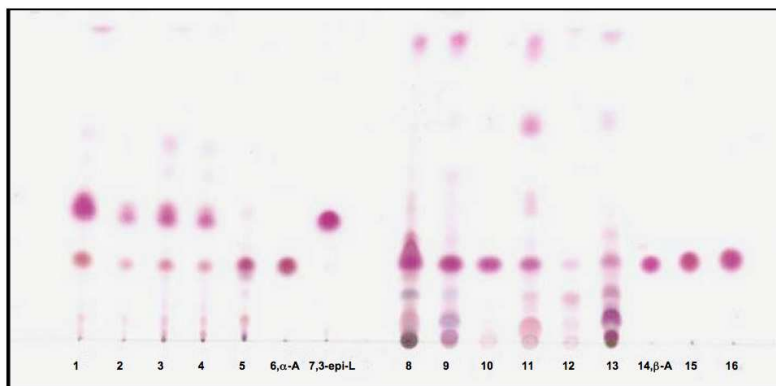


Fig. 2. TLC plate. Tracks: 1=MCT; 2=MCS; 3=MCN; 4=MCP; 5=MER; 6 and 15= $\alpha$ -amyrin; 7=3-*epi*-lupeol; 8=Dandelion; 9=Olive; 10=Cancerina; 11=Nance wastes; 12=Bearberry; 13=Pot marigold; 14= $\beta$ -amyrin; 15= $\alpha$ -amyrin and 16=mixture  $\alpha$ -,  $\beta$ -amyrin.

### 2.1.3 Analysis

Gas chromatography (CG) is applied to determine the concentration of  $\alpha$ -,  $\beta$ -amyrin and  $\alpha$ -,  $\beta$ -amyrin mixtures. The chemical composition of the essential oil of Lemon Catnip (*Nepeta cataria* L. var. *citriodora* Balbis) was determined by CG-MS, and  $\alpha$ -amyrin was detected in the hydrodistilled volatile of Lemon Catnip (Wesolowska et al., 2011).  $\alpha$ -amyrin has also been determined in the kernel fats of the shea tree (*Vitellaria paradoxa*; Sapotaceae) of sub-Saharan countries (Akihisa et al., 2010). CG of the apolar extract from *Clusia Minor* L. leaves led to the identification of 25 compounds, lupeol and  $\alpha$ -amyrin being the most abundant triterpenoids (Mangas-Marin et al., 2008). CG-MS fingerprints for cerumen from the stingless bee *Tetragonula carbonaria* in South East Queensland, Australia, showed trace quantities of TMS ethers of  $\beta$ -amyryns (Massaro et al., 2010). Studies on the constituents of yellow Cuban propolis by CG-MS revealed the presence of large amounts of triterpenic alcohols including  $\beta$ -amyrin, (Márquez-Hernández et al., 2010). Solid-phase extraction and GC-MS were developed to separate and enrich only sterols from unsaponifiables of vegetable, hazelnut and olive oils, detecting sterols, lupeol and  $\delta$ -,  $\beta$ -amyrin (Azadmard-Damirchi et al., 2010). Epicuticular and intracuticular waxes from both adaxial and abaxial surfaces of *Kalanchoe daigremontiana* leaves (Hamet et Perr. De la bathie) were analyzed by CG. All wax mixtures were found to contain triterpenoids and fatty acids, the triterpenoid fraction containing small amounts of  $\beta$ -amyrin (van Maarseveen & Jetter., 2009). Fatty acid, phytosterol, and polyamine conjugate profiles of corn edible oils were analyzed by GC-MS and HPLC, and a few minor sterols and  $\beta$ -amyrin were identified and quantified using GC-FID (Moreau et al., 2009). Fatty acids, phytosterols and tocopherols of Milk thistle (*Silybum marianum*) seeds were determined in four varieties grown in Ardebil-Iran. In this study using TLC-GC, dimethylsterols were predominant followed by cycloartenol and  $\beta$ -amyrin, (Fathi-Achachlouei, Azadmard-Damirchi., 2009). CG proved an effective method for quantitative measurement of the  $\beta$ -sitosterol content of white mulberry (*Morus alba*) leaves and bark without derivatization. Sterols, lupeol and  $\alpha$ -,  $\beta$ -amyrin were identified in leaves and bark by GC-FID analysis (Böszörményi et al., 2009). GC and CG-MS were used to

analyse hexane extracts from seven oleoresins of *Protium* species. High concentrations of  $\alpha$ - and  $\beta$ -amyryn were identified in *P. strumosum* (64%) and *P. tenuifolium* (66.7%) (Silva et al., 2009). Finally, the analytical performances of three atmospheric-pressure sources, electrospray (ESI), atmospheric-pressure chemical ionization (APCI) and atmospheric-pressure photoionization (APPI), were evaluated for the analysis of pentacyclic triterpenes in liquid chromatography-mass spectrometry (LC-MS) (Zarrouk et al., 2010). The developed LC-MS method was used to characterize pentacyclic triterpenes in tree plant extracts. The main component of birch bark was betulin and the extracts of Okume resin exhibited high amounts of  $\alpha$ - and  $\beta$ -amyryn (Rhourri-Frih et al., 2008). Other technique used to quantitate and determine amyryns is Reversed-Phase High Performance Liquid Chromatography (RP-HPLC). HPLC was used for analysis of some isomeric plant triterpenoids ( $\alpha$ -amyryn and  $\beta$ -amyryn  $\delta$ -amyryn, lupeol, lupenon, lupeol acetate, cycloartenol acetate, ursolic acid oleanolic acid and two sterols) (Martelanc et al., 2009), other studies was for analysis of medicinal plants and Mexican Copal resins (Hernández-Vázquez et al., 2010) and resin obtained from species of the genus *Protium* (Burseraceae) (Dias et al., 2011).

#### 2.1.4 Sources of $\alpha$ -, $\beta$ -amyryns

$\alpha$ -amyryn is a triterpene of natural origin isolated from various sources, most notably plant resins. Considerable amounts (up to g/kg) of this triterpene are available in the resins of *Bursera* and *Protium* species of the *Burseraceae* family. Other known sources of  $\alpha$ -amyryn include Mexican copal (5 g/kg) (Hernández-Vázquez, et al., 2010), *Cassia obtusifolia* (140 mg/kg) (Sob et al., 2010) and the resin of *Commiphora holtziana* (syn. *Commiphora erythraea*) (200 mg/kg) (Manguero, et al., 2009). The most important sources of  $\beta$ -amyryn include lotus (*Nelumbo nucifera* Gaertn) bee pollen (3 g/kg) (Xu. et al., 2011), bark of "cuachalalate" (*Amphipterygium adstringens*) (2.4 g/kg) (Rosas-Acevedo et al., 2011), semi-preparative isolation from resin of *Protium* ( $\alpha$ -amyryn 1g /kg and  $\beta$ -amyryn 1.7 g/Kg) (Dias et al., 2011), *Eucalyptus globulus* biomass residues from the pulping industry (326 mg/kg) (Domingues et al., 2010), *Ficus carica* latex (1.2 g/kg) (Oliveira et al., 2010), root bark of *Ficus cordata* (20 mg/kg), steam bark *Ficus cordata* (200 mg/kg) (Kuetze et al., 2008) and leaves and bark of *Byrsonima crassa* Niedenzu (IK) (1.3 g/kg) (Higuchi et al., 2008). Mixtures of  $\alpha$  and  $\beta$ -amyryn were obtained from steam bark residues of *Byrsonima crassifolia* (Nance) (9 g/kg) (Hernández-Vázquez et al., 2010), leaves of *Byrsonima fagifolia* Niedenzu (2.3 g/Kg) (Higuchi et al., 2008) and leaves of *Pouteria gardnerii* (Mart & Miq) Bahemi gave  $\alpha$ -,  $\beta$ -amyryn and other triterpenes (Silva et al., 2009).

Plants reported since 2008 to possess  $\alpha$ -amyryn,  $\beta$ -amyryn and a  $\alpha$ ,  $\beta$ -amyryn mixture in minor amounts (detected and isolated) are listed here.  $\alpha$ -Amyryn has been isolated from the resin of *Boswellia carterii* Birdw (Wang et al., 2011), detected in stemwood and bark from *Populus x euramericana* (Xu et al., 2010), isolated (65 mg/kg) from the *n*-hexane extract of the leaves of *Melastoma malabathricum* L (Sirat et al., 2010), identified in the methanol extract of the stem bark of *Poncirus trifoliata* (Feng et al., 2010), isolated (1 mg/kg) from the methanol extract of the stem bark of the African tree *Antiaris Africana* Engler (Vouffo et al., 2010), detected in seed oil of Saskatoon berries (*Amelanchier alnifolia* Nutt.) (Bakowska-Barczak et al., 2009), isolated (23 mg/kg) from the methanol extract of stem bark and leaves of *Ficus pandurata* Hance (Ramadan et al., 2009), dried rhizomes of *Nelumbo nucifera* (Chaudhuri et al., 2009), and detected in bread wheat (Nurmi et al., 2008).

| Plant   | $\alpha$ -amyrin | $\beta$ -amyrin | $\alpha/\beta$ -amyrin | Ref                            |
|---|------------------|-----------------|------------------------|--------------------------------|
| Mexican copal   | 5g/Kg            |                 |                        | Hernandez-Vazquez et al., 2010 |
| <i>Cassia obtusifolia</i>                                       | 0.14g/kg         |                 |                        | Sob et al., 2010               |
| <i>Commiphora holtziana</i> (syn. <i>Commiphora erythraea</i> ) |                  |                 |                        | Manguro, et al., 2009.         |
| <i>Nelumbo nucifera</i> Gaertn                                  |                  | 3g/kg           |                        | (Xu. et al., 2011)             |
| <i>Amphipterygium adstringens</i>                               |                  | 2.4g/kg         |                        | Rosas-Acevedo et al., 2011     |
| <i>Protium</i> sp   | 3.1g/kg          | 1.7g/Kg         |                        | Dias et al., 2011              |
| <i>Eucalyptus globulus</i>                                      |                  | 0.3g/kg         |                        | Domingues et al., 2010         |
| <i>Ficus carica</i>   |                  | 1.2g/Kg         |                        | Olivera et al 2010             |
| <i>Ficus cordata</i>  |                  | 0.2g/Kg         |                        | Kuete et al., 2008             |
| <i>Byrsonima crassa</i> Niedenzu (IK)                           |                  | 1.3g/kg         |                        | (Higuchi et al., 2008.         |
| <i>Byrsonima crassifolia</i> (Nance)                            |                  |                 | 9g/kg                  | Hernández-Vázquez et al., 2010 |
| <i>Byrsonima fagifolia</i>                                      |                  |                 | 2.3g/Kg                | Higuchi et al., 2008           |
| <i>Pouteria gardnerii</i> (Mart & Miq)                          |                  |                 | X                      | Silva et al., 2009             |

Table 2. List of selected materials containing  $\alpha$ -,  $\beta$ -amyrin and  $\alpha/\beta$ -amyryns

$\beta$ -Amyrin has been isolated and detected in various materials: an ethanolic fraction of oleo-gum-resin from *Ferula gummosa* (Jalali et al., 2011), the plant *Carpobrotus edulis*, (Martins et al., 2011), the leaves of *Clerodendrum inerme* (L.) (22.5 mg/kg) (Parveen et al., 2010), the chloroform and ethyl acetate fractions of the methanolic extract of *Carpobrotus edulis* (Martins et al., 2010), chloroform extract of aerial parts of the plants or calli of *Euphorbia tirucalli* L. (Uchida et al., 2010), seed oil of *Capparis spinosa* (Tlili et al., 2011), chloroform extract of the leaves of *Ficus benjamina* (var. *camosa*) (Moraceae) (Simo et al., 2009), leaves (2 mg/kg) of *Pyrenacantha staudii*, (Falodun et al., 2009), ethanol extract of leaves of *Olea europea* L. (Wang et al., 2009), ethyl acetate extract of apple peels of the Red Delicious variety (*Malus domestica* Borkh) (He et al., 2008), air-dried leaves of *Tectona philippinensis*, an endemic and endangered Philippine medicinal plant (Ragasa et al., 2008), a mixed benzene and chloroform extract of leaves of *Rhus alata* (Parveena et al., 2008), and an extract of stem bark of *Piptadenia Africana*, a western Cameroon plant (Mbouangouere et al., 2008).

An  $\alpha$ - and  $\beta$ -amyrin mixture has been detected in the following plants: *n*-hexane and chloroform extracts of the epicuticular wax layer of *Mandevilla guanabaria* and *Mandevilla moricandiana*, (Cordeiro et al., 2011), an ethanolic extract of roots of *Salacia amplifolia*, (Wang et al., 2011), and chloroform extracts of Blue Honeysuckle (*Lonicera caerulea* L.) (Palíková et al., 2008).

A multitude of extraction and isolation schemes have been used for the procurement of  $\alpha$ -amyryn,  $\beta$ -amyryn and an  $\alpha/\beta$  amyryn mixture. Typically, dry material (resins, leafs and stem barks) is extracted with hexane or another non-polar solvent, (Fig. 3), and the resulting extract is directly subjected to column or thin layer chromatography. An alternative procedure is sequential fractionation by silica gel columns using various solvents. The

amyryns are not readily visible on TLC plates UV ( $\lambda = 254$  and  $365$  nm) but are easily detected following exposure to iodine vapors, anisaldehyde- $H_2SO_4$  or vanillin- $H_2SO_4$  spray reagents.

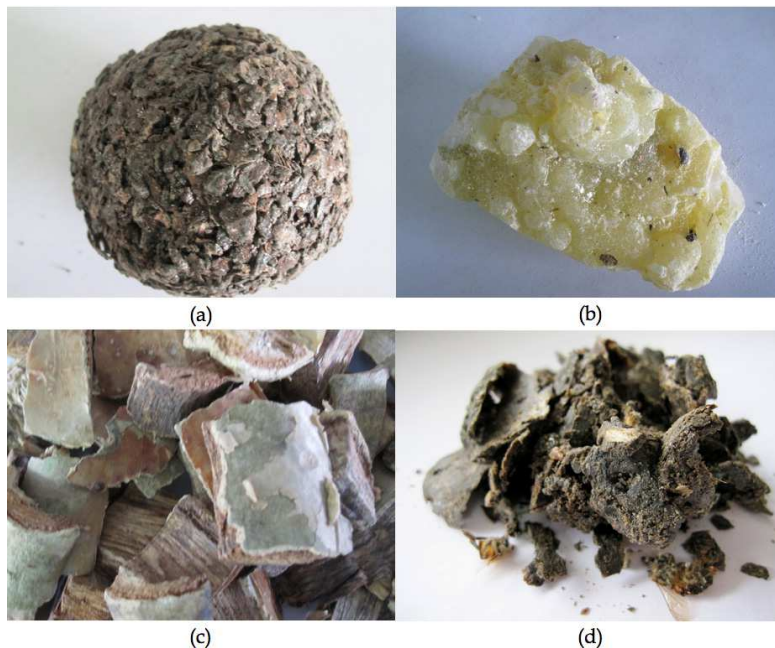


Fig. 3. Sources of amyryns; a) Copal Piedra, b) White Copal, c) Bursera bark, d) Propolis

## 2.2 An overview of pharmacological activities of $\alpha$ , $\beta$ -amyryns

$\alpha$ ,  $\beta$ -amyryns have been shown to exhibit various pharmacological activities *in vitro* and *in vivo* conditions against various health-related conditions, including conditions such as inflammation, microbial, fungal, and viral infections and cancer cells.

### 2.2.1 Anti-microbial and anti-fungal

The antimicrobial properties of *n*-hexane and methanol extracts of *Bombax malabaricum* flowers were examined against different bacterial, fungal and yeast strains. The methanol extract was highly active against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Neisseria gonorrhoea*, *Pseudomonas aeruginosa* and *Candida albicans*, whereas the *n*-hexane extract displayed moderate-to-weak activities against the same test microorganisms. An *n*-hexane extract afforded sterols including  $\alpha$ -amyryn (El-Hagrassi et al., 2011). A bioassay-guided fractionation of *n*-hexane extracts of *Bursera simaruba* (L) Sarg. leaves resulted in the isolation and identification of five sterols and  $\beta$ -amyryn. Additionally, *n*-hexane extracts have displayed anti-inflammatory activity on adjuvant-carrageenan-induced inflammation in rats (Carretero et al., 2008).  $\alpha$ -Amyryn and other compounds have been proposed as possible biomarkers for the fungal resistance of grape-vine leaves (*Vitis vinifera*) (Batovska et al., 2008).

$\beta$ -Amyrin has been found to exhibit antifungal and antimicrobial activity against some microbes. The antifungal activity of *Melia azedarach* L. leaves was investigated against *Ascochyta rabiei* (Pass.) Lab., the cause of destructive blight disease of chickpea (*Cicer arietinum* L.). Bioassay-guided fractionation revealed that the chloroform fraction of the methanolic extract of *M. azedarach* leaves was highly effective against *A. rabiei*. Six compounds, namely  $\beta$ -sitosterol (1),  $\beta$ -amyrin (2), ursolic acid (3), benzoic acid (4), 3,5-dimethoxybenzoic acid (5) and maesol (6), were isolated from this fraction. All compounds showed antifungal activity,  $\beta$ -amyrin being the most effective, with an MIC value of 0.0156 mg mL<sup>-1</sup> (Jabeen et al., 2011).

In a recent study on the leaves of *Siraitia grosvenorii*,  $\beta$ -amyrin and other bioactive compounds were obtained, and their activities against the growth of oral bacterial species *Streptococcus mutans*, *Actinobacillus actinomycetemcomitans*, and *Fusobacterium nucleatum* and the yeast *C. albicans* were evaluated *in vitro*.  $\beta$ -amyrin only exhibited a slight inhibition of *Streptococcus mutans* and *Fusobacterium nucleatum* (Zheng et al., 2011). Bioassay-guided fractionation of the methanol extract of the stem bark of *Klainedoxa gabonensis* Pierre ex Engl. (Irvingiaceae) afforded 12 compounds: four flavonoids and eight (including  $\beta$ -amyrin) triterpenes. Antimicrobial activities in the triterpenoids ranged from low to non-existent (Wansi et al., 2010). In this study, the *in vitro* antibacterial activity of the methanolic extract and isolated compounds from the bark of *Byrsonima Crassifolia* against twelve bacteria and the yeast *C. albicans* was investigated. Eight known compounds,  $\beta$ -amyrin, betulin, betulinic and oleanolic acid, quercetin, epicatechin, gallic acid and  $\beta$ -sitosterol, were isolated and evaluated for their antimicrobial activity. Bacterial growth was inhibited by  $\beta$ -amyrin, oleanolic and gallic acid at concentrations ranging from 64 to 1088  $\mu$ g.mL<sup>-1</sup> (Rivero-Cruz et al., 2008).

### 2.2.2 Anti-inflammatory activity

Hexane extracts of *Bursera simaruba* (L.) Sarg. leaves display an anti-inflammatory effect on adjuvant-carrageenan-induced inflammation in rats. In order to isolate and identify the active compounds of the hexane extract, we performed a preliminary phytochemical study and a bioassay-directed fractionation using the carrageenan-induced paw oedema test in mice. From the nine fractions (A-I) obtained, A and E showed the strongest anti-inflammatory activity, comparable to that of the reference drug phenylbutazone. Sterols and  $\alpha$ -amyrin have been isolated and characterized from these fractions, the evidence suggesting that these bioactive compounds may play a key role in the anti-inflammatory effects of *B. Simaruba* extracts (Carretero et al., 2008).

*Ligustrum* (privet) plants are used by Chinese physicians to prevent and cure hepatitis and chronic bronchitis. Three common *Ligustrum* plant spp., namely *Ligustrum lucidum* Ait. (LL), *L. pricei* Hayata (LP) and *L. sinensis* Lour. (LS) were collected to assess their analgesic/anti-inflammatory effects on chemical-induced nociception and carrageenan-induced inflammation in rodents. The methanol extracts from *Ligustrum* plant leaves effectively inhibited nociceptive responses induced by 1% acetic acid and 1% formalin. LP and LL reduced the edema induced by 1% carrageenan. The most potent *Ligustrum* plant was LP, which also reduced abdominal Evans blue extravasations caused by lipopolysaccharide, lipoteichoic acid, autocrines and sodium nitroprusside. The triterpenoid content of the three



*Ligustrum* spp. was measured by HPLC, the highest content of  $\beta$ -amyrin, betulinic acid and lupeol being found in LP. This work suggested that these three triterpenoids are responsible for the anti-inflammatory potency of LP (Wu et al., 2011).

A recent report describes that the roots of *Calotropis gigantea* (Linn.) R.Br, traditionally used in India to treat asthma, possess anti-lipoxygenase activity, it was found that intraperitoneal administration of indomethacin did not block edema formation, but edema was inhibited by montelukast and methanolic extracts of *C. gigantea* roots. This result indicates that the extract from *C. gigantea* was responsible for the inhibition of the lipoxygenase pathway in the arachidonate metabolism. Therefore, it can be concluded that *C. gigantea* may have a similar mechanism of action as dexamethasone as well as antioxidant and anti-lipoxygenase effects, possibly due to the presence of  $\alpha$ -amyrin and  $\beta$ -amyrin (Bulani et al., 2011).

Aqueous and organic extracts of *Acacia visco* Lor. Ap Griseb (Fabaceae) were tested for anti-inflammatory activity in experimental rat models. The extracts revealed an anti-inflammatory effect against carrageenan-induced oedema, phospholipaseA-induced oedema, and cotton pellet-induced granuloma without any acute toxic effects. Among the class of compounds characterized from *A. visco* leaves, the triterpenoids lupeol,  $\alpha$ -amyrin and  $\beta$ -amyrin may be mainly responsible for these anti-inflammatory properties (Padernera et al., 2010).  $\alpha$ -,  $\beta$ -Amyrin ameliorates L-arginine-induced acute pancreatitis in rats. It has been demonstrated that the crude resin of *Protium heptaphyllum* (March.) has an  $\alpha$ - and  $\beta$ -amyrin ratio of 63:37. The mixture of both compounds and methylprednisolone treatments significantly ( $P < 0.05$ ) attenuated the L-arginine-induced increases in pancreatic wet weight/body weight ratio, and decreased the serum levels of amylase and lipase, and TNF- $\alpha$  and IL-6, in comparison with the vehicle control. Also, pancreatic levels of MPO activity, TBARS, and nitrate/nitrite were significantly lower. The conclusion of this study is that  $\alpha$ ,  $\beta$ -amyrin has the potential to combat acute pancreatitis by acting as an anti-inflammatory and antioxidant agent (Melo et al., 2010).

Another study has shown the systemic preventive or therapeutic anti-inflammatory action of the triterpenes  $\alpha$ - and  $\beta$ -amyrin in TNBS-induced colitis in mice. It was found that  $\alpha$ -,  $\beta$ -amyrin is as efficacious as dexamethasone in reversing the macroscopic and microscopic outcomes of TNBS-induced colitis, including the restoration of cytokine balance. Furthermore, the results also indicate that inhibition of NF- $\kappa$ B and CREB activation is certainly the main mechanism through which these triterpenes exert their anti-inflammatory action (Vitor et al., 2009). Another report demonstrated for the first time that  $\alpha$ -,  $\beta$ -amyrin isolated from *Protium heptaphyllum* modulates acute periodontal inflammation in rats by reducing neutrophil infiltration, oxidative stress and the production of proinflammatory cytokine TNF- $\alpha$ , and suggests that these triterpenes might be useful as a therapeutic agent for the treatment of gingivitis and to retard the progression of periodontitis (Holanda-Pinto et al., 2008).

### 2.2.3 Other pharmacological activities

$\alpha$ - and  $\beta$ -Amyrin have been tested for a variety of other biological activities. An anti-ulcer effect of *Cyrtocarpa procera* and *Amphipterygium adstringens* was assayed on experimental gastric injury in rats and phytochemical analysis allowed the identification of  $\beta$ -amyrin and  $\beta$ -sitosterol in *A. adstringens* (Rosas-Acevedo et al., 2011). The triterpenoids  $\beta$ -amyrin,

cohulupone and garcinielliptone were isolated from the pericarp, heartwood and seed of *Garcinia subelliptica*, respectively, and the three compounds showed an inhibitory effect on xanthine oxidase. Treatment of NTUB1, a human bladder cancer cell, with  $\beta$ -amyryn or  $\beta$ -amyryn in cotreatment with cisplatin for 24 h resulted in a reduced viability of cells. This work suggested that  $\beta$ -amyryn exhibited weak cytotoxic activities against NTUB1 cells (Lin et al., 2011). The antiproliferative effects of *n*-hexane, chloroform and aqueous methanol extracts prepared from the whole plant of *Centaurea arenaria* M.B. ex Willd. were investigated against cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF7) and skin epidermoid carcinoma (A431) cells, using the MTT assay. Only the flavonoids and lignans showed moderate activity against these cell lines and  $\beta$ -amyryn was inactive (Csapi et al., 2010). From the ethyl acetate fraction of the stem bark of *Camellia japonica*, three new triterpenoids, 3- $\beta$ -O-acetyl-16 $\beta$ -hydroxy-12-oxoolean, 3- $\beta$ -O-acetyl-16 $\beta$ -hydroxy-11-oxoolean-12-ene, and 3- $\beta$ -O-acetyl-16 $\beta$ -hydroxyolean-12-ene, along with seven known compounds, 3- $\alpha$ -hydroxy-1-oxofriedelan, friedelin, 3- $\beta$ -friedelanol, canophyllol, 3-oxofriedelan-1(2)-ene,  $\beta$ -amyryn, camellenodiol, and camelledionol, were isolated. Their structures were established on the basis of spectroscopic analysis and chemical evidence. The isolated compounds were tested *in vitro* for their cytotoxic activities against the A549, LLC, HL-60 and MCF-7 cancer cell lines. Among them,  $\beta$ -amyryn exhibited weak cytotoxicity against A549 and HL-60 cancer cell lines with IC<sub>50</sub> values of 46.2 and 38.6  $\mu$ M, respectively (Thao et al., 2010). Another report showed that the methanol extract obtained from soxhlet extraction of leaves of *Ardisia elliptica* Thunberg (Myrsinaceae) contained  $\alpha$ - and  $\beta$ -amyryn, determined by GS-MS. The leaf extract inhibited platelet aggregation with an IC<sub>50</sub> value of 167  $\mu$ g/mL, using bioassay guided fractionation.  $\beta$ -Amyryn was isolated and purified showing an IC<sub>50</sub> value of 4.5  $\mu$ g/mL, while that of aspirin was found to be 11  $\mu$ g/mL, indicating that  $\beta$ -amyryn is more potent than aspirin in inhibiting collagen-induced platelet aggregation (Ching et al., 2010). Two triterpenes,  $\beta$ -amyryn and 12-oleanene 3 $\beta$ , 21 $\beta$ -diol, were isolated as a mixture from the chloroform soluble fraction of an ethanolic extract of *Duranta repens* (Verbanaceae) stem. The mixture was highly effective against the larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae) as a mosquitocide. *C. quinquefasciatus* is a potential vector of *Wuchereria bancrofti* (Filarioidae), the causative agent of human lymphatic filariasis (Nikkon et al., 2010). One study has examined the potential trypanocidal activity of different plant species growing in the Brazilian Cerrado, after *in vitro* screening of 20 extracts obtained from 10 plants. The phytochemical analysis of the most active extracts (hexane extracts) allowed the identification of  $\beta$ -amyryn,  $\alpha$ -amyryn, lupeol and other triterpenes and sterols. The results showed that pure amyryns are inactive whereas the *n*-hexane leaf extract of *Tibouchina stenocarpa* cogn. Melastomataceae was active. The trypanocidal activity of the extract may be due to the presence of other compounds (Cunha et al., 2009).

### 3. Conclusion

$\alpha$ - and  $\beta$ -Amyryn are bioactive compounds commonly found in leaves, barks and resins. Such plant material is an interesting source of these triterpenoids, as it allows for easy extraction. Extensive research over the last four years has identified  $\alpha$ - and  $\beta$ -amyryn in several plants and the pure compounds have shown anti-microbial, anti-inflammatory and other interesting biological activities. Amyryns are also involved in the biosynthetic

pathways of other biologically active compounds such as avenacine, centellosides, glycyrrhizin or ginsenosides. The development of biotransformation systems to convert amyrins into these or other compounds would open new ways for using  $\alpha$ - and  $\beta$ -amyrins as a source of bioactive plant secondary metabolites more scarcely distributed in the plant kingdom. In this context, the bioconversion of  $\alpha$ -amyrin into centellosides in *Centella asiatica* cell cultures has been recently reported (Hernandez-Vazquez et al., 2010).

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#### 5. References

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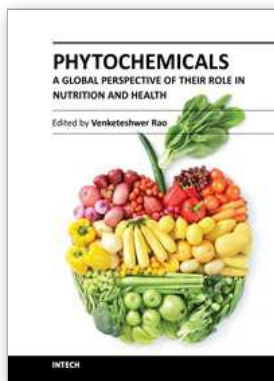
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## **Phytochemicals - A Global Perspective of Their Role in Nutrition and Health**

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Phytochemicals are biologically active compounds present in plants used for food and medicine. A great deal of interest has been generated recently in the isolation, characterization and biological activity of these phytochemicals. This book is in response to the need for more current and global scope of phytochemicals. It contains chapters written by internationally recognized authors. The topics covered in the book range from their occurrence, chemical and physical characteristics, analytical procedures, biological activity, safety and industrial applications. The book has been planned to meet the needs of the researchers, health professionals, government regulatory agencies and industries. This book will serve as a standard reference book in this important and fast growing area of phytochemicals, human nutrition and health.

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