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# A review of amygdalin characteristics

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#### Abstract

This review prefaces the current state of knowledge in the field with the history and evolution of amygdalin. Information and scientific data were summarized from the literature on the amygdalin content of certain fruit seeds and seeds, the methods for their determination, the controversies on the one hand and the toxicity due to its enzymatic degradation, on the other possible anticancer activity of the amygdalin

Keywords: amygdalin, cyanogenic glycosides, fruit kernels, toxicity, anticancer, analytical methods HPLC

#### 1. Introduction

Known as an ideal solution against cancer, vitamin B 17 or laetrile has an evolution full of scientific disputes. History amygdalin begins in the year 1850 when it was used in the treatment of cancer. Research has shown that the metabolism of amygdalin results in highly toxic cyanide derivatives. Based on this idea, biochemist Ernest T. Krebs laid the foundations in 1950 of a semisynthetic molecule with a structure identical to that of amygdalin, which he named laethrile or vitamin B17. Based on this idea, biochemist Ernest T. Krebs laid the foundations in 1950 for a semi-synthetic molecule with a structure identical to that of amygdalin, which he named laethrile or vitamin B17, because Krebs planned for laethrile to be labeled a nutritional supplement. In this way, try to circumvent the law tangentially on drugs trade [24].

American Cancer Society did not confirm laethrile as a vitamin because it did not scientifically define the vitamin, ie it was not that chemical compound that was argued to be crucial for maintaining human health [18].

Amygdalin is a cyanogenic glycoside that can also be found in commercial networks, which is in considerable quantities in the seeds and kernel fruits of the *Rosaceae* family. The amygdalin consists of mandelonitrile lyase and gentiobiosis. It is decomposed into hydrocyanic acid, benzaldehyde and D-glucose due to the amygdalin, prunasine and mandelonitrile hydrolysis [32].

Amygdalin (D-mandelonitrile- $\beta$ -D-glucoside-6- $\beta$ -D-glucoside) belongs to the group of toxic plant cyanogenic glycosides and the plant material in which amygdalin is found in considerable quantities consists of apple seeds, apricot kernels, almonds, cherries, peaches and plums [14].

Amygdalin has its plant source, and its molecular formula is  $C_{20}H_{27}NO_{11}$ , consisting of one unit of hydrocyanic acid (HCN), one unit of benzaldehyde and two units of glucose. Amygdalin as such is nontoxic, but its amount of hydrocyanic acid decomposed by some enzymes is a poisonous substance, but it has some protective effects on plants.

Pharmacological studies have shown that amygdalin has many bioactivities, such as renal fibrosis and angiogenesis inhibition, endocrine regulators, antiatherosclerotic, gastroprotective and immunomodulatory effects, neuroprotective and neuritis activities [26]. Due to its anti-tumor and anti-cancer potential, amygdalin has become a growing attraction for the medical world and scientists.

Figure 1. Chemical structure of amygdalin

# 2. Amygdalin - vegetable sources, toxicity and activity anticancer

In the evolution of mankind, almonds have been one of the most important nutrients for human food and health due to its high nutritional value. It has been researched that some cyanogenic glycosides such as prunasine and amygdalin, contained in almond leaf and fruit, are used in the pharmaceutical and medical industries [29].

Amygdalin is found in many species of the Rosaceae family (especially in the subfamilies Prunoideae and Maloideae) and species of the families Leguminosae, Gramineae, Araceae, Compositae and Euphorbiaceae [6, 29]. Widespread in nature, in the seeds of some plants, amygdalin is abundant in the seeds and kernel fruit species such as almonds, apricots and plums of the subfamily Prunoideae [3, 22].

Research has been conducted on the chemical composition and use of unripe peaches as functional materials [12]. Peach kernels have also been studied for their use in the prevention of atherosclerosis and have been processed into functional products. Amygdalin (D-mandelonitrile-β-D-glucoside-6-β-D-glucoside) is defined as a cyanogenic glycoside contained in considerable plant toxin. concentrations in the apple seeds and, apricot, almond, cherrie and peache kernels and in quantities large in plum kernels [14].

Amygdalin, also known as "laetryl" and "vitamin B17", has been recognized over the years by proponents of natural medicine and much research in the field highlights the anticancer effects of amygdalin [5]. It is one of the most debated

vitamins in the last three decades, becoming a controversial topic for scientists. It is a cyanogenic plant glycoside belonging to the Rosaceae family also found in plants such as lime beans, clover and sorghum [5, 21].

Peaches (Prunus persica L. Batsch, Rosaceae) although native to China, they are widely grown in various parts of the world, especially in colder climates. The peach fruit develops from a single ovary that ripens into a fleshy pulp, with a juicy outside, making up the edible part of the fruit inside which is the woody and hard kernels. The pulp of the fruit adheres firmly to the kernel, which makes it difficult to separate the fruit from the kernel [13]. Usually, peaches have a sweeter white pulp and less acidity than those with a yellow pulp, which usually have an acidic, slightly astringent taste, similar to that of sweetness. Climatic conditions, cultivation techniques, variety and differences between them were some of the factors that affected the phytochemical content of the fruit [13].

Due to the medicinal benefit, there is a growing demand for fruits with kernels, including peaches, but there have been problems with the processing of these seeds, which is why concerns have been raised about resolving these issues. There are studies that have observed various important physicochemical properties of these fruit seeds for health benefits. Such parameters can vary greatly between different varieties [13].

Dried mature seeds of Armeniaca sibirica L., Armeniaca mandshurica (Maxim.) And Armeniaca vulgaris Lam. are wild apricot kernels that usually have a bitter taste, due to the presence of amygdalin. Amygdalin, the cyanogenic diglucoside D-mandelonitrile-β-D gentiobiozide, is generally present in apricot kernels, bitter almond kernels and the seeds of other fruits of the genus Prunus [32]. Scientific studies have shown potential toxicity dangerous due to the hydrolysis of amygdalin resulting in hydrocyanic acid (HCN), a substance toxic to the human body. Ingestion of apricot kernels in relatively large quantities can thus cause cyanide poisoning [17].

At present, studies on the extraction, identification and pharmacological effects of amygdalin have focused mainly on the core of the kernels of Prunus amygdalus, *Prunus armeniaca* [7]. Prunus persica, Prunus salicina, but less Eriobotrya japonica fruit [26]. Apricot kernels of the Prunus Tomentosa Thunb family. are considered traditional Chinese

herbs that contain amygdalin as the main effective ingredient. The core of the apricot kernels is the dried and ripe seed of Prunus armeniaca L. and Prunus armeniaca L. var. Ansu Maxim. It has been prescribed in many traditional Chinese medicines as an antitussive, with expectorant and laxative functions. Prunus Tomentosa Thunb. is the dried and mature seed of Prumus humilis Bge., Prunus japonica Thunb. and Prunus pedunculata Maxim [25, 27]. Armeniacae Semen, seed Prunus armeniaca Linne var. ansu Maximowicz, which belongs to the Rosaceae family, contains amygdalin and appreciable amounts of vegetable oil. This seed has been widely used in oriental medicine for the treatment of cough, respiratory tract, relief of constipation and sore throat [11, 31].

Amygdalin content of four species of fruit trees with kernels (apricot, peach, plum and bitter apricot) in various stages of cultivation and development, was identified by the method of thin layer chromatography. The results showed a much higher amygdalin content in the kernels than in the thighs, roots, shoots or leaves. It should be noted that the largest amount of amygdalin was found in bitter apricot kernels [32]. Edible vegetable oil, benzaldehyde, activated carbon, amygdalin and hydrocyanic acid are extracted from apricot kernels. Known as cyanogenetic glycoside (cyanogen amygdalin, D (-) - mandelonitrile-gentiobioside), it is found abundantly in many tissues and especially in Prunus seeds (cherry, peach, almond and apricot) [30].

Amygdalin, as a cyanogenic glycoside, has also been identified in bitter almonds, apricot kernels and the seeds of other Prunus species. Amygdalin has been reported to be used to treat all cancers, although the effects of treatment with it have not been proven by rigorous and accurate scientific and medical research. On the other hand, there are some reported studies that indicate that amygdalin can be dangerous to human health [28].

The most common cyanogenic glycoside in fruits and fruit kernels is amygdalin. The content of the fruit seeds in the amygdalin varies significantly from seed to seed, depending on their specific characteristics. The apple (Malus domestica) is the most prized fruit in the Rosaceae family. Apples can be eaten raw or made into an alcoholic (cider) or non-alcoholic beverage (apple juice) or apple sauce.

Although apples are rich sources of vitamins and other nutrients, apple seeds contain high levels of cyanogenic glycosides [4].

Plants containing cyanogenic glycosides occur in about 2,000 species of 110 families (eg, Rosaceae, Poaceae, Papilionaceae, Euphorbiaceae, Scrophulariaceae), including many edible plants and fruit seeds, such as peaches and edible almond kernels. The amygdalin content usually increases during the fruit development stage and remains constant or decreases minimally during ripening. In peach seed, the amygdalin content is higher in the endocarp than in the mesocarp. The bitter taste in almond kernel is determined by the content of amygdalin (cyanogenic diglucoside) [10].

Amygdalin has a wide range of pharmacological activities, including analgesic and antiasthmatic effects. The enzymatic hydrolysis of amygdalin produces benzaldehyde and hydrocyanic acid, while its acid hydrolysis produces a single gentibiosis product. Due to its prehepatic metabolism, amygdalin generates another enzyme in the gut, namely prussine [19].

This has been a topic of debate with scientific questions about the veracity of tumor regression due to amygdalin. Controversies are also being debated about the toxicity of amygdalin at the right doses. Based on these premises, many scientists place amygdalin between a toxic chemical substance and a therapeutic substance as a drug. Many in vitro experiments have shown that it is capable of inducing apoptotic cell death and stopping the cell cycle or delaying its proliferation in many cancer cells [8, 16].

Seeds containing cyanogenic glycosides, consumed in excessive amounts can cause acute or chronic toxicity to humans and animals because amygdalin hydrolyzed by the action of b-glucosidase, decomposes into benzaldehyde, glucose and hydrocyanic acid, and hydrocyanic acid is the substance that has an effect toxic and fatal to human health [29].

As such, amygdalin is not a toxic substance, but the properties of hydrocyanic acid resulting from the enzymatic hydrolysis of amygdalin indicate its toxicity. Research into the therapeutic effects of amygdalin has highlighted its effective properties and its importance in treating diseases [15, 23].

Amygdalin has analgesic effects due to the presence of benzaldehyde in its molecule. However, its anticancer properties remain controversial and considered research topics in perspective. However, the use of amygdalin as a cancer drug has been called into question due to its toxicity to normal cells and its limited pharmacokinetic properties [2].

Due to the repeated failures of clinical trials, many authors have challenged the role assigned to amygdalin, namely that of therapeutic drug. Research into the toxicity of amygdalin has been shown to be toxic, especially when taken orally. This led to the FDA banning amygdalin in 1979. Anaerobic bacteria present in the intestinal tract are able to release cyanide into the intestine following the breakdown of amygdalin [9].

However, rhodanese, which is a ubiquitous enzyme found in the mitochondria of many organisms, is able to convert the high content of cyanide from amygdalin into thiocyanate, a toxic harmless substance [19].

Recently, the content of amygdalin and prunasine (cyanogenic monoglucoside) but also the enzymes involved in the composition of amygdalin have been studied in four different genotypes of almonds. Researchers in the field have suggested distinct cellular locations of the enzymes responsible for the degradation process, involving prunasine hydrolase. Another point of view is a possible source of bitter taste in almonds from the seed coat [1].

3. Analytical methods determination of amygdalin

Analytical metods		Sources	Ref
HPLC	detector: SPD-10AVvp diode array detector (lmax = 238 nm), system controller: SCL-10 Avp. Pump: LC-6AD, column: ACE 5-C18 (250 · 4.6 mm, 5 mm), mobile phase: CH3CN:H2O (75:25, v/v), flow rate: 0.9 mL·min–1, injection volume: 20 mL, column temperature: 25 C. Amygdalin (Sigma-Aldrich Inc., USA) was used as a standard. Concentrations of amygdalin were determined by correlation of their HPLC peak areas to their calibration curves. The concentrations were expressed as g·kg–1. All chemical analyses were carried out in triplicates	almond kernels	[29]
reversed–phase HPLC-UV	A Shimadzu HPLC consisting of a 20ADXR pump, SIL-20ACXR autosampler, degasser, and SPD-M20A diode array detector set at 214 nm was used. The column was a Phenomenex C18, Type Nucleosile 3, 120 A (150 mm ê 4.60 mm, 3 μm) placed in a column oven set at 40°C. The mobile phase consisted of methanol and water (25:75, v:v) and the flow rate was 1 ml/min. The mobile phase was sonicated (20 min, 22°C ± 2°C) to remove gas bubbles before use. The injection volume was 5 μl.	green plum, apricot, black plum, peach, red cherry, black cherry and almond kernels	[3]
HPLC	HPLC instrument (LC-10A, Shimadzu Co., Kyoto, Japan). The HPLC column was a SupelcosilTM LC-18-S (φ 4.6×250 mm, Supelco, Bellefonte, PA, USA), and the samples were analyzed at a UV wavelength of 210 nm with 20% methanol (0.7 mL/min) as the mobile phase (17). The amygdalin contents were determined with linear regression methods using an amygdalin standard curve.	peach cultivars at different fruit development stages (seed, endocarp and mesocarp)	[14]

HPLC	Conditions for the HPLC were: detector, Waters, US/M996, 717 plus photodiode array detector; column, $\mu$ Bondapak C18 10 $\mu$ m 125A; mobile phase, 25% CH3OH; column temperature, 30°C; flow rate, 1.0 mL/min; UV detector, 214 nm; injection volume, 10 $\mu$ L. The amygdalin content was determined by comparing the obtained peaks with the standards ones according to their relative rise and time. Samples were run in duplicate.	unripe peach, Japanase apricot	[13]
Second-derivative spectrophotometry	Absorption spectra were measured using a Shimadzu recording spectrophotometer, model UV-1700, over a scanning range of 250–275 nm with a high scanning speed and a derivative wavelength difference of $\Delta\lambda$ =2 nm. Amplitudes were recorded between 266 nm and 268 nm.	wild apricot	[17]
HPLC	Quantification was performed using a C18 reversed phase column (250 mm $\times$ 4.6 mm, 5.0 $\mu$ m) at 25 °C using a Hitachi L-2300 column oven. Chromatography was performed using a flow rate of 0.8 ml/min, a 15:85 acetonitrile:water mixture as the eluent, 20 $\mu$ l of sample, and with detection under UV at 214 nm using Hitachi L2455 DAD detector. All analyses were performed in triplicate	wild apricot	[17]
HPLC	Conditions for the HPLC were: Detector: SPD-10AVvp diode array detector (max = 238 nm), system controller: SCL-10 Avp, Pump: LC-6AD, Column: ACE 5-C18 (250 x 4.6 mm) 5, mobile phase: 75% ACN, flow rate: 0.9 ml/min, injection volume: 20 l, column temperature: 25°C. Amygdalin (Sigma) was used as a standard. The amygdalin content was determined by comparing the obtained peaks with the standards according to their relative rise and time.	sweet and bitter apricot kernels	[30]
HPLC	the HPLC system Agilent 1200 model equipped with a UV-VIS detector on a C18 Supelcosil column 250 x 4.5 x 5 µm. It was used as eluent methanol/water 15/85 in isocratic system at a flow rate of 1 ml/min for 26 minutes. Chromatogram was recorded at wavelength 215 nm.	apricot, plum and peach kernels	[20]

## 4. Conclusion

Research has shown inconclusive results on the therapeutic effects of amygdalin, and therefore more studies are needed to elucidate the pharmacological mechanisms of amygdalin in terms of optimal dose of use, the possibility of combining amygdalin with other antitumor treatments.

Research and testing of chemical synthesis of the active substance amygdalin have been suggested in order to increase its antitumor effect and reduce its adverse effects for clinical use.

Controversies related on the one hand to the toxicity of amygdalin due to its enzymatic degradation, on the other hand to potential anticancer activities, should generate further research in this field in order to elucidate all the questions raised by scientists.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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