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Bioactive profile of pumpkin: an overview on terpenoids and their health-promoting properties

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Pumpkins belong to the family Cucurbitaceae, a medicinally and economically important plant group. In fact, some of them are cultivated both for their edible fruits than for the cure of a wide array of health-related problems. Cucurbitaceae produce the so-called cucurbitacins, important compounds with curative effects. Furthermore, they are responsible for the bitter taste and the toxicity of the plants. In the cucurbits, and in particular in the pumpkins, there is a significant quantity of terpenoid-metabolites with remarkable biological properties. Among the most represented terpenoids, there are triterpenes, to which cucurbitacins and other similar molecules belong, tetraterpenes represented essentially by carotenoids, and some sesquiterpenes. This review is focused on the investigation of the main Cucurbitaceae terpenes, together with related biological and health activities.

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Introduction

Pumpkin belongs to Cucurbitaceae which include pumpkin, gourds, melons and squashes [1,2]. *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata* represent the most important species of Cucurbitaceae cultivated in the world. Overall, pumpkin is grown for its fruit and edible seeds. Pumpkin plant has large and simple leaves while the fruit is more fibrous and less sweet than winter squash [3]. Seed vary in size depending upon variety and type [4], but generally big, abundant and edible. Pumpkin seeds are pressed to extract oil (approximately 50% of the content), which is used for cooking [5] and cosmetics. The seeds are also consumed as toasted, while both fruits and the internal parts of pumpkin are commonly consumed as vegetable. The oil is rich with oleic and linoleic acids, Δ7-sterols (avenasterol, spinasterol), and Δ5-sterol (sitosterol, stigmasterol), important active [50]. It also contains remarkable bioactives such as triterpenoids, sesquiterpenoids, tetraterpenoids (carotenoids), tocopherols, and polyphenols [6•,7]. In particular, the main phenolic classes detected in pumpkin seeds are flavonoids, phenolic acids and lignans [8]. Saponins are also present, together with cucurbitacins that have been isolated from the plant [9••]. The carotenoid β-carotene is present in all of the species, and associated with considerable biological properties, the main one being the precursors of vitamin A (antioxidant) found in various fruits and vegetables [10]. Besides the capacity to improve the immune function [11] and reduce the risk of heart disease and cancer [12,13], the seeds and its oils have been used to treat enlarged prostate gland [14]. In fact and as previously reported [15] oral administration of pumpkin seed oil (2–4 mg/100 g for 20 days) inhibited the testosterone-induced hyperplasia of the prostate, producing a beneficial effect on the hyperplastic prostate gland. Several studies have focused on the use of natural products and botanicals to prevent and treat cancer and other related diseases. Among bioactive compounds from natural sources, an important group are triterpenes that show cytotoxic properties against tumor cells [16,17]. Terpenoids represent a large class of chemical compounds, present in fair quantities in the fruit and the seeds. In particular, a specific class of triterpenoids, the cucurbitacins, have been extracted from fruit and pumpkin seeds. Cucurbitacins, and the other considered triterpenoids, showed many relevant biological/pharmacological properties [18•] and at this time it is one of the most studied classes of bioactive compounds. The current review aims to summarize the studies on a particular group of bioactive compounds, the terpenoids, to improve knowledge concerning new terpene structures and their related healthy properties.

Triterpenoids' profile

Triterpenes constitute a group of molecules not strictly related to each other and biogenetically derived from

active isoprene [19]. Molecules at 30 carbon atoms, such as squalene, represent the main precursors of triterpenes, and by means of cyclization and oxidation, various structures are formed. The basic structure of triterpenes is built from six isoprene units. The most common chemical structures of triterpenes are shown in Figure 1, including pentacyclic-oleanane, ursane, taraxerane, taraxastane, lupane, and tetracyclic-dammarane and cucurbitane.

Cucurbitacins

Cucurbitaceae produce the so-called cucurbitacins, which are responsible for the curative effects. Furthermore, these compounds are the reason for the bitter taste and toxicity of the plants. Cucurbitacins are a class of highly oxygenated compounds, with a tetracyclic triterpenic structure derived from the cucurbitane skeleton [19-(10→9β)-abeo-l0α-lanost-5-ene] also known as 9β-methyl-19-nor lanosta-5-ene (Figure 2). These particular kind of terpenoids differ from most of the other tetracyclic triterpenes in the high degree of unsaturation and for the presence of numerous keto-groups, hydroxy-groups and acetoxy-groups. These compounds are classified into twelve categories, involving cucurbitacins A-T. From a chemical point of view, these molecules differ with respect to oxygen functionalities at various positions (Table 1). Besides of the free forms, cucurbitacins are also present in glycosidic forms, as β-2-monoglycosides, with the sugar moiety represented by D-glucose or L-rhamnose such as in cucurbitacin B glucoside.

Cucurbitacins can act as digestive and purgative agents being rich in strong bitter principles [20] able to stimulate the gastric secretion [9••]. Generally, cucurbitacins are not used as medical agents because of their toxicity, but in some cases, they have demonstrated the potential for treating different pathologies including inflammation, cancer, or autoimmune diseases. At this regard, many pharmacological and clinical investigations have shown that cucurbitacin B (CuB) and cucurbitacin E (CuE) (Figure 3) possess various pharmacological activities, such as antioxidant, antimicrobial, antiulcer, antitumor, anti-hepatitis and anti-hyperglycemic effects [21]. In particular, Cucurbitacin E caused disruption of the cytoskeleton structure of actin and vimentin inhibiting the proliferation of prostate cancer cells [22]. Moreover, cucurbitacins also inhibited proliferation of endothelial cells accompanied by a disruption of the F-actin and tubulin microfilaments cytoskeleton, normal mitogen-induced T-lymphocytes [23] and reduced cell motility suggest an anti-angiogenesis and anti-metastasis role for cucurbitacins. In addition is capable of inducing and maintaining high proliferation rates in lymphocytes [24].

The main biological and pharmacological effects of cucurbitacins, from studies of the last 5 years, are reported in Table 2.

Cucurbitacin E and cucurbitacin E glycoside: studies of toxicity

Experiments with cucurbitacins carried out on laboratory animals have shown acute toxic effects. Cucurbitacin E and cucurbitacin E glycoside, which are the most common cucurbitacins identified in plant foods, have oral LD50 values in mice of 340 and 40 mg/kg body weight, respectively [9••]. The main toxicological effect produced by cucurbitacin D in experimental animals appears to be an increase in capillary permeability, irritation of the intestinal mucosa, and a strongly increased intestinal motility. Overall, in all the experiments, the animals die from congestion of the intestine, pancreas, liver and kidneys. Significant consequences occur at the pulmonary level where lungs became oedemateous and contain large amounts of fluids. Even at the thoracic and abdominal levels, it is possible to find appreciable quantities of liquids [9••].

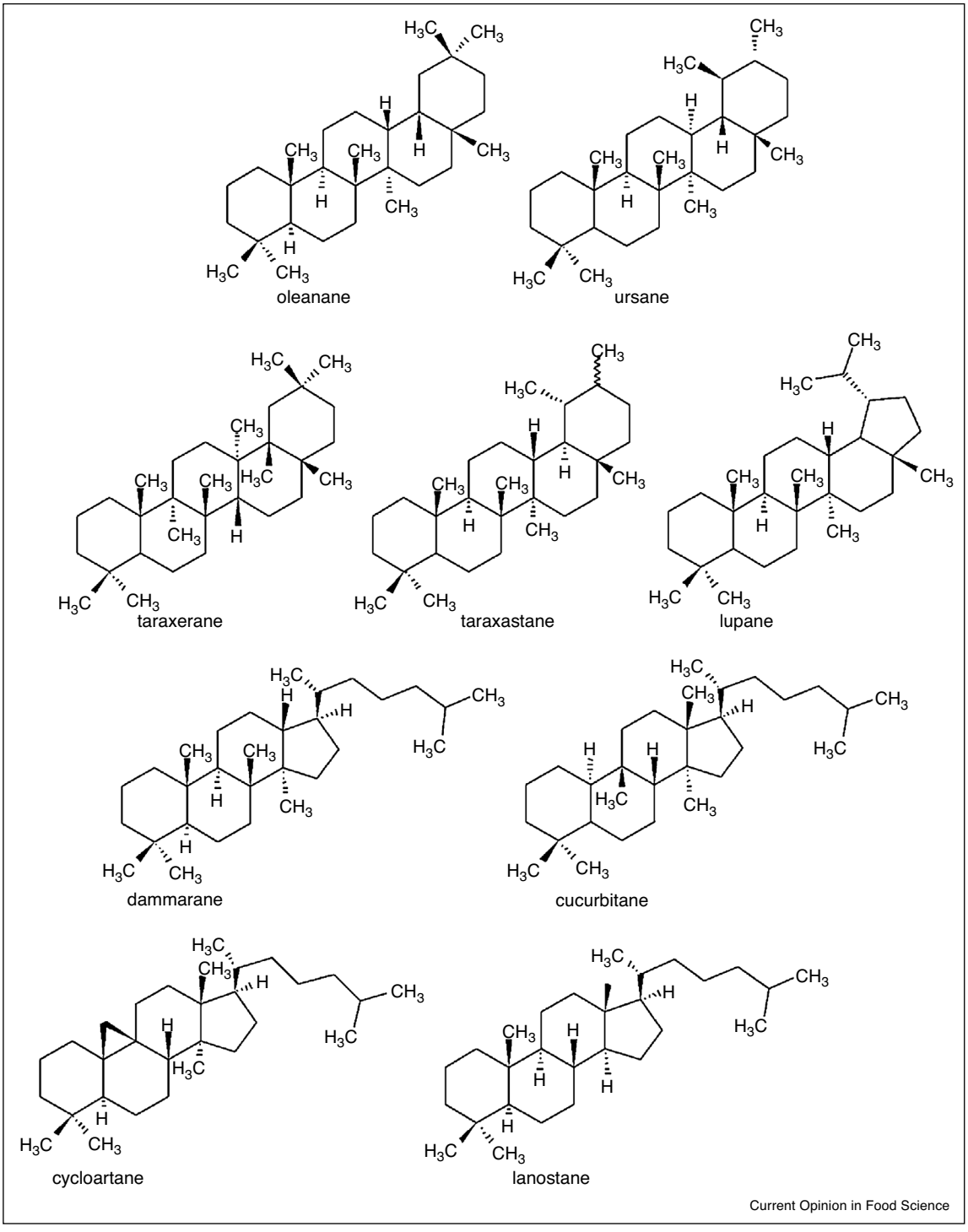
Other triterpenoids

Recently, six triterpenes belonging to the multiflorane-type triterpene esters, were isolated from pumpkin (*C. maxima*) seeds. Of these, three were new: 7α-hydroxy-multiflor-8-ene-3α,29-diol 3-acetate-29-benzoate (a), 7α-methoxymultiflor-8-ene-3α,29-diol 3,29-dibenzoate (b), and 7β-methoxymultiflor-8-ene-3α,29-diol 3,29-dibenzoate (c), while the other three were already known [39••]. Moreover, three new triterpene esters from pumpkin (*C. maxima*) seeds, were extracted and fully characterized [40••]. A novel 3α-p-Nitrobenzoylmultiflora-7:9(11)-diene-29-benzoate and two new triterpenoids from the seeds of zucchini (*Cucurbita pepo* L) were isolated and fully characterized [41••]. Nevertheless, in ancient literature several triterpenes from pumpkin were also described [42,43]. Furthermore, pumpkin oil is rich in squalene (3.53 g/kg), an important linear triterpene involved in sterols synthesis, with relevant antioxidative properties, that contribute to oxidative stability of the pumpkin oil [44].

Tetraterpenoids: β-carotene, lutein and b-cryptoxanthin

Carotenoids are natural pigments within tetraterpenoid group, which are metabolized by plants, algae, and photosynthetic bacteria and are responsible for the yellow, orange, and red colors in various fruits and vegetables [45]. These pigments possess a multitude of functions [46,47]. Carotenoids have pro-vitamin A activity, especially β-carotene [48] which presents 100% of this activity. Vitamin A is essential for night vision, growth, development, and maintenance of the epithelial tissue, besides having immunologic function [49]. In the same way, the antioxidant β-carotene in pumpkins helps to improve the immune function, reducing also the risk of heart disease and cancer [50]. Lycopene is one of most studied carotenoid [51], present in pumpkin at low concentration, and a lot of biological/

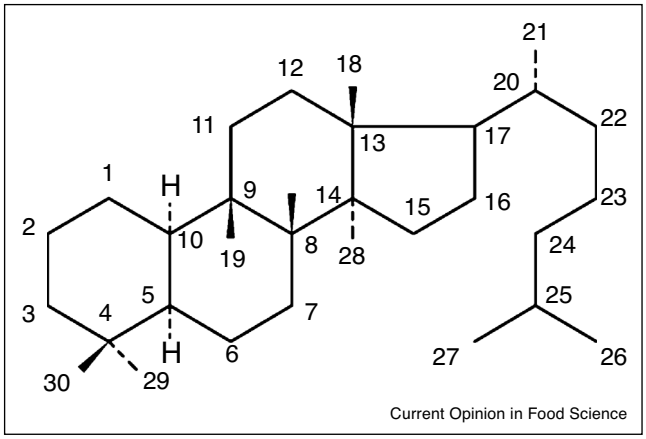
Figure 1



Main triterpenes structures.

pharmacological effects are reported [52,53]. Furthermore, xanthophylls, such as lutein and zeaxanthin, normally present in Cucurbitaceae, have been detected by many researchers in various plants and vegetables [54,55] both in free and esterified form. Xanthophylls are positively related to several healthy effects, such as the ability to treat the age-related macular degeneration (AMD) [56,57].

Figure 2



Cucurbitane skeleton.

Diterpenes

Few data are available in recent literature regarding diterpene compounds in pumpkin. Two new entkaurane-type diterpene glycosides were extracted from zucchini (*Cucurbita pepo* L.) seeds [58].

Sesquiterpenes

Sesquiterpenes, have attracted significant interest because of the roles they play in biological systems and their utility for human uses [59]. The basic structure of these compounds is very different by the other terpenes, in fact a first structural diversity lies in the assembly of the 15-carbon skeletons making up the backbone of all sesquiterpenes and then the layering of functional groups and substituents upon the structural scaffolds in distinct regio-specific and stereospecific manners [60]. Sesquiterpene-like structures are scarcely mentioned in the works reviewed [50,61].

Conclusions

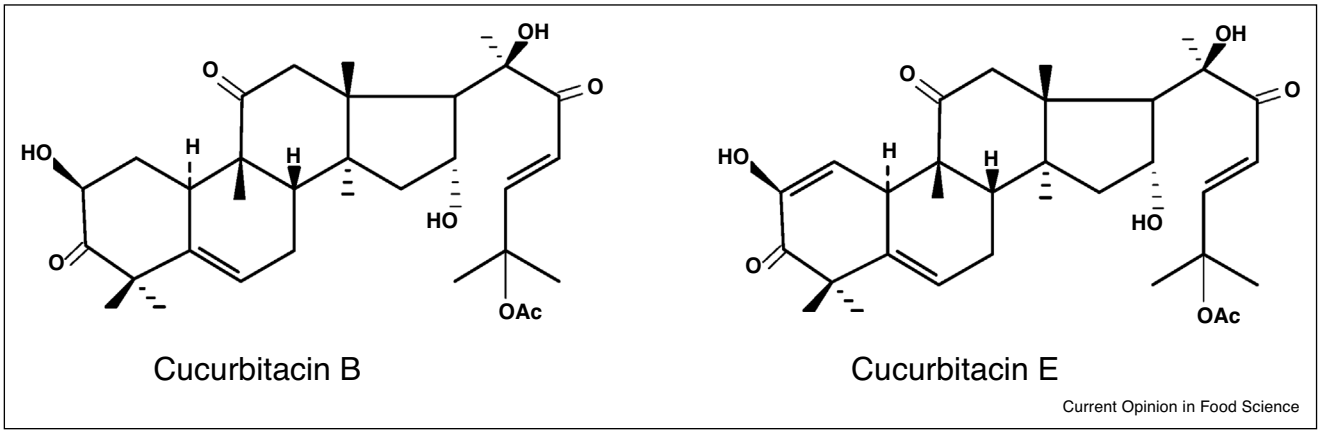
The medicinal value of pumpkin lies in chemical compounds that are able to produce a specific physiological effect in human body. From this point of view, by reviewing the studies on the chemical, biological and pharmacological characteristics of the pumpkin, considering both seeds and fruits, it can certainly be stated that

Table 1

Main cucurbitacins by chemical modification of cucurbitane skeleton [9••]

Name	Chemical group on carbon									Double bonds		
	2	3	11	16	19	20	22	24	25			
Cucurbitacin A	OH_β	=O	=O	OH_α	OH	OH_β	=O		OAc		Δ_5	Δ_{23}
Cucurbitacin B	OH_β	=O	=O	OH_α		OH_β	=O		OAc		Δ_5	Δ_{23}
Cucurbitacin C		OH	=O	OH_α	OH	OH_β	=O		OAc		Δ_5	Δ_{23}
Cucurbitacin D	OH_β	=O	=O	OH_α		OH_β	=O		OH		Δ_5	Δ_{23}
Cucurbitacin E	OH_β	=O	=O	OH_α		OH_β	=O		OAc	Δ_1	Δ_5	Δ_{23}
Cucurbitacin I	OH_β	=O	=O	OH_α		OH_β	=O		OH	Δ_1	Δ_5	Δ_{23}
Cucurbitacin J	OH_β	=O	=O	OH_α		OH_β	=O	OH_α	OH	Δ_1	Δ_5	
Cucurbitacin K	OH_β	=O	=O	OH_α		OH_β	=O	OH_β	OH	Δ_1	Δ_5	
Cucurbitacin L	OH_β	=O	=O	OH_α		OH_β	=O		OH	Δ_1	Δ_5	

Figure 3



Chemical structures of cucurbitacin B and E.

Table 2

Elucidations on the main biological and pharmacological activities of *Cucurbitaceae* triterpenes

Key activity	Reference
Cucurbitacins were proposed as new powerful tool to treat metabolic disease and implicate STAT3 as a new target for the development of functional foods	[25••]
Cucurbitacin IIb was shown to possess anti-inflammatory activity through modulating multiple cellular behaviors and signaling pathways	[26••]
Cucurbitacin E was proposed as potential clinical tool for the prevention or treatment of human breast cancer	[27]
Cucurbitacin B showed anticancer activity against hypoxia-inducible factor-1 (HIF-1) activation	[28]
Cucurbitacin I, D, and E were shown to present a potent cytotoxic activity and are metabolized as sulfate and glucuronide conjugates	[29]
Cucurbitacin E may cause complex drug-drug interactions, due to its dual functional regulator of CYP3A and P-glycoprotein activities	[30••]
Cucurbitacin I was suggested to have value as an adjunct chemotherapy agent	[31]
Cucurbitacins inhibition targets are JAK2/STAT3 signaling pathway, rupture of the cytoskeletal actin and vimentin networks	[32]
Cucurbitacin B showed potent chemopreventive activity for prostate cancer, inhibiting also ACYL signaling in human cancer	[33]
The triterpene extract of *Cucurbita maxima* seeds showed melanogenesis inhibitory activity, wound healing and anti-inflammatory properties	[34]
Cucurbitacin D induces cell cycle arrest and apoptosis by inhibiting STAT3 and NF-κB signaling in human breast carcinoma cells	[35••]
Cucurbitacin B suppressed non-small-cells lung (NSCL) cancer growth *in vitro* and H1299 xenograft *in vivo*	[36]
Cucurbitacin B in combination with curcumin could serve as a novel and promising approach for human hepatoma, reversing multidrug resistance and enhancing apoptosis induction	[37]
Triterpenes are involved in the inhibition of enzymes involved in glucose metabolism, preventing the development of insulin resistance and normalizing plasma glucose	[38]

the bioactive substances, and in particular the terpenoid compounds, are of considerable interest for human health. Their properties can be exploited against a variety of diseases thus implying their pharmacological and nutraceutical uses.

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Conflict of interest

None declared.

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