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REVIEW ARTICLE

Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects

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Tannins are a heterogeneous group of high MW, water-soluble, polyphenolic compounds, naturally present in cereals, leguminous seeds and, predominantly, in many fruits and vegetables, where they provide protection against a wide range of biotic and abiotic stressors. Tannins exert several pharmacological effects, including antioxidant and free radical scavenging activity as well as antimicrobial, anti-cancer, anti-nutritional and cardio-protective properties. They also seem to exert beneficial effects on metabolic disorders and prevent the onset of several oxidative stress-related diseases. Although the bioavailability and pharmacokinetic data for these phytochemicals are still sparse, gut absorption of these compounds seems to be inversely correlated with the degree of polymerization. Further studies are mandatory to better clarify how these molecules and their metabolites are able to cross the intestinal barrier in order to exert their biological properties. This review summarizes the current literature on tannins, focusing on the main, recently proposed mechanisms of action that underlie their pharmacological and disease-prevention properties, as well as their bioavailability, safety and toxicology.

LINKED ARTICLES

This article is part of a themed section on Principles of Pharmacological Research of Nutraceuticals. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v174.11/issuetoc

Abbreviations

GSE, grape seed extract; HTs, hydrolyzable tannins; NOAEL, no-observed-adverse effect level; OPCs, oligomeric proanthocyanidins

Tables of Links

TARGETS		
Other protein targets[a]	**Enzymes**[f]	MMP-9
Bax/Bcl-2	Akt (PKB)	Glycosidase
TNF-α	Amylase	HATs
GPCRs[b]	Caspase 1	Lipase
CCR2	Caspase 3	Lipoxygenases
Nuclear hormone receptors[c]	CDKL	Proteases
PPARγ	ERK-1 (p44 MAPK)	
Transporters[d]	ERK-2 (p42 MAPK)	
GLUT4	MAPKs	
Catalytic receptors[e]	MAPK 14 (p38 MAPK)	
NLRP3	MMP-2	

LIGANDS	
Endothelin-1	IL-1β
GLP-1	L-cysteine
Glutathione	L-homocysteine
IL-10	VCAM-1
IL-12	

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d,e,f]Alexander *et al.*, 2015a,b,c,d,e,f).

Introduction

Tannins are a heterogeneous group of water-soluble polyphenolic compounds of high molecular weight (500–3000 Daltons) – with as many as 20 hydroxyl groups – and are present in plants, foods and beverages (de Jesus *et al.,* 2012). Being phenolic compounds, tannins are chemically reactive and form inter- and intra-molecular hydrogen bonds that are able to interact with, and precipitate macromolecules, such as proteins and carbohydrates. They are also responsible for the astringent taste of many fruits and vegetables (de Jesus *et al.,* 2012; Lamy *et al.,* 2016). With one to five hydroxyl groups, astringency is increased, while from seven groups upwards, it decreases, given that steric hindrance starts to counterbalance the strength of the hydrogen bonds (Zou *et al.,* 2015).

Tannins can be classified into two groups: hydrolysable tannins and condensed tannins (also named catechin tannins or proanthocyanidins). Hydrolysable tannins can be further divided into gallotannins, which provide sugar and gallic acid on hydrolysis, and ellagitannins, which on hydrolysis do not yield just sugar and gallic acid but also ellagic acid (see Lamy *et al.,* 2016). These compounds, as the name suggests, are hydrolyzed by weak acids and decomposed by high temperatures to yield pyrogallol, a hepatotoxic and highly irritant compound (Jiménez *et al.,* 2014).

The second class, condensed tannins, also referred to as proanthocyanidins, is the most abundant plant-derived polyphenols. They are oligomers of flavan-3-ol (catechin monomers) and/or flavan-3,4-diol, usually linked by C-C (4–8 or 6–8) and occasionally by C-O-C bonds with a wide structural diversity (de Jesus *et al.,* 2012; Lamy *et al.,* 2016) and are also called oligomeric proanthocyanidins (OPCs). These compounds are not readily hydrolyzed; they decompose in acidic alcoholic conditions giving red pigments called phlobaphenes. To date, though, the chemistry of proanthocyanidins is only partly known.

Despite their abundance in our diet (estimated daily intake 0.1–0.5 g), tannins have received little attention, probably due to their polymeric nature and high structural complexity (Serrano *et al.,* 2009). In recent years, considerable attention has been paid to proanthocyanidins and their monomers due to the potential beneficial effects on human health, including immunomodulatory, anti-inflammatory, anticancer, antioxidant, cardio-protective and antithrombotic properties (Nile and Park, 2014; Smeriglio *et al.,* 2014a; Sieniawska, 2015). However, to assess their precise role in human health, it is essential to gather further knowledge on their pharmacological and toxicological behaviour. In fact, although many plant-derived proanthocyanidins are widely used now as nutritional supplements, evidence of their safety and potential for long-term toxicity is still lacking (Berry *et al.,* 2016).

Here we have reviewed the latest developments and knowledge on the occurrence, dietary intake and biological effects of tannins, with particular attention on the pharmacological and toxicological aspects.

Chemical features and sources

Tannins (proanthocyanidins and hydrolysable tannins) are one of the main secondary metabolites found in cacao beans, tea, wines (mainly red), fruits, juices, nuts, chocolate, legumes and cereal grains (Table 1). There are many ways to define tannins based on their properties, solubility and presence of substituents (Okuda and Ito, 2011). Taking into account, for instance, the structural features of hydrolysable tannins, they can be identified as follows: gallotannins, ellagitannins and complex tannins.

Proanthocyanidins. Proanthocyanidins are oligomers or polymers of flavan-3-ols, where the monomeric units are

Table 1

Main tannin families and related food sources

	Species	Tannins (mg·100 g⁻¹ or mg·100 mL⁻¹)		
		Proanthocyanidins	Ellagitannins	Gallotannins
Fruits				
Cranberries	*Vaccinium oxycoccus* L.	194–496	—	—
Chokeberries	*Aronia melanocarpa* (Michx.) Elliott	553–2106	—	—
Plums	*Prunus domestica* L.	32–334	—	—
Black diamond	*Prunus spp*	210–267	—	—
Blueberries	*Vaccinium myrtillus* L.	87–274	—	—
–	*Vaccinium corymbosum* L.	311–335	—	—
Black currants	*Riges nigrum* L.	105–255	3–6	—
Red currants	*Ribes rubrum* L.	30–61	—	—
Blackberries	*Rubus fructicosus* L.	5–46	150–270	—
Crowberries	*Empetrum nigrum* L.	153–173	—	—
Lingonberries	*Vaccinium vitis-idaea* L.	175–545	—	—
Red Grapes	*Vitis Labrusca* L.	8–75	—	—
Grape seeds	*Vitis Vinifera* L.	2180–6050	—	—
Strawberries	*Fragaria × ananassa* Duchesne	15–183	71–83	—
Peaches	*Prunus persica* L.	29–110	—	—
Apricot	*Prunus armeniaca* L.	8–73	—	—
Raspberries	*Rubus occidentalis* L.	3–74	160–326	—
Pears	*Pyrus communis* L.	5–81	—	—
Apple	*Malus domestica* Borkh.	46–278	—	—
Pomegranate	*Punica granatum* L.	—	58–177	—
Guava	*Pisidium spp.*	—	20–25	—
Mango	*Mangifera indica* L.	—	—	30–160
Juices				
Cranberry	–	20–21	—	—
Apple	–	52–69	—	—
Grape	–	45–47	—	—
Nuts				
Almonds	*Prunus dulcis* (Mill.) D.A.Webb	67–257	—	20–34
Hazelnuts	*Corylus spp.*	125–645	—	—
Pecans	*Carya illinoinensis* (Wangenh.) K.Koch	238–695	11–33	—
Pistachio nuts	*Pistacia vera* L.	113–271	—	—
Walnuts	*Juglans regia* L.	35–87	36–59	—
Legumes				
Beans	*Phaseolous vulgaris* L.	5–830	—	—
Carob fibre	*Ceratonia siliqua* L.	1–19	—	—
Cowpeas	*Vigna unguiculata* (L.) Walp.	17–319	—	—
Lentils	*Lens culinaris* Medik.	1–2	—	—
Peanuts	*Arachis hypogaea* L.	121–141	—	—
Cereal grains				
Barley	*Hordeum vulgare* L.	59–153	—	—
Buckwheat	*Fagopyrum esculentum* Moench	4–46	—	—
Sorghum	*Sorghum bicolor* (L.) Moench	413–5333	—	—
Rice	*Oryza sativa* L.	9–91	—	—

(continues)

Table 1 (Continued)

| | Species | Tannins (mg·100 g^{-1} or mg·100 mL^{-1}) | | |
		Proanthocyanidins	Ellagitannins	Gallotannins
Beverages				
Wine	–	1–53	2–5	—
Tea	–	1–5	—	—
Cacao beans	*Theobroma cacao* L.	6100–8100	—	—
Chocolate	–	828–1332	—	—

linked mainly by C-4 → C-8 bonds, although less frequently also C-4 → C-6 linkages can be found. These types of linkages lead to the formation of the so-called B-type proanthocyanidins. A-type proanthocyanidins, on the other hand, are characterized by an additional bond between C-2 → C-7 of the basic flavan-3-ol units (Figure 1). Proanthocyanidins are composed of different flavan-3-ol subunits, known as proanthocyanidin monomers or catechins. The most common monomeric units are the diastereomers of (epi)catechin, (epi)afzelechin and (epi) gallocatechin. In this class of flavonoids, two aromatic homocyclic rings (A and B) and a heterocyclic ring (C) are present, and they are joined by a three-atom carbon bridge. Their C ring features a hydroxyl group at the C-3 position,

and it lacks a double bond between C-2 and C-3. The most abundant proanthocyanidins in vegetables are those composed of catechin and epicatechin units (procyanidins), while much less common are propelargonidin or prodelphinidin containing (epi)afzelechin or (epi) gallocatechin basic units (Figure 1), present in foods such as barley, broad beans, red kidney beans, redcurrants, pinto beans, black tea and cinnamon (Landete, 2011; de Jesus *et al.*, 2012; Mateos-Martín *et al.*, 2012; Lamy *et al.*, 2016). Procyanidins have been identified, in natural sources, as dimers (e.g. procyanidin B1-B8, procyanidin A1-A2), trimers (e.g. procyanidin C1 and C2, selligueain A and B), tetramers and oligomeric structures with a degree of polymerization ranging from 5 to 11. The most common acyl substituent
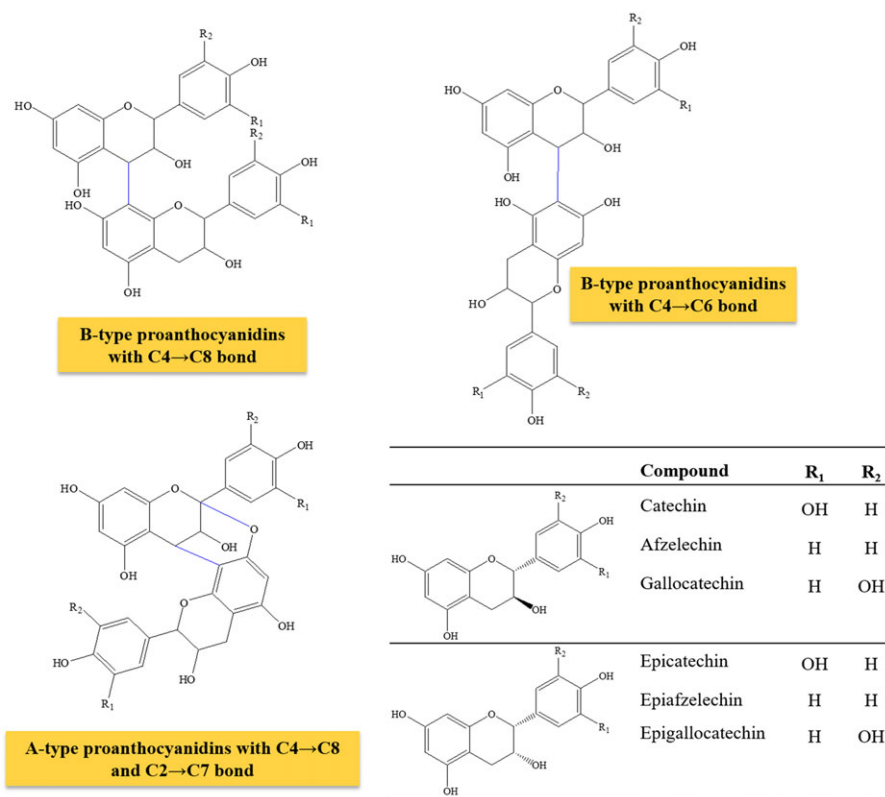

Compound	R$_1$	R$_2$
Catechin	OH	H
Afzelechin	H	H
Gallocatechin	H	OH
Epicatechin	OH	H
Epiafzelechin	H	H
Epigallocatechin	H	OH

Figure 1
Basic chemical features of B- and A-type proanthocyanidins and their monomeric units.

(as in the case of tea- or wine-derived species) is a galloyl group bound to the hydroxyl group in the C-3 position. The carbohydrate moieties are usually linked to the C-3 or the C-5 position in glycosylated proanthocyanidin oligomers.

Gallotannins. Gallotannins represent the simplest class of hydrolyzable tannins (HTs), containing gallic acid substituents esterified with a polyol residue (mainly D-glucose). The β anomer of glucose is the dominant configuration, although rare examples of the α configuration can be found in natural derivatives. The glucose hydroxyl groups can be partly or totally substituted by phenol units to produce initially β-glucogallin (1-*O*-galloyl-β-D-glucopyranose). The biosynthetic pathway, starting from this molecule and after the galloylation reaction, yields di-, tri-, tetra, penta-, hexa-, hepta- and octagalloylglucoses. Of these, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose is usually employed as the prototypical molecule of gallotannins (Figure 2). The formation of *meta*- or *para*-depside bonds is characteristic of these molecules and involves esterification of the aromatic hydroxyl group rather than those present in aliphatic structures. However, many other derivatives can be found, for example gallotannins with 10 or more (up to 12) units of gallic acid esterified to a single glucose moiety, as in the case of tannins obtained from sumac (*Rhus semialata*) or oak galls (*Quercus infectoria*), utilized since ancient times to tan animal hides to produce leather. Although glucose is by far the most abundant polyol identified in tannins, glucitol, fructose, shikimic acid, xylose, hamamelose, saccharose, quercitol and quinic acid may constitute the core molecules for the subsequent galloylation processes. However, these derivatives are not very common and have only been isolated in maple, chestnut and witch hazel.

Ellagitannins. Different from gallotannins (rarely found in nature), ellagitannins are present in many plant families, and almost 500 molecules have been isolated and identified to date (Okuda and Ito, 2011; Lamy *et al.,* 2016). Although not all ellagitannins are hydrolyzable, they continue to be classified as hydrolyzable tannins for historical reasons. They can be found in numerous forms: monomeric (e.g.
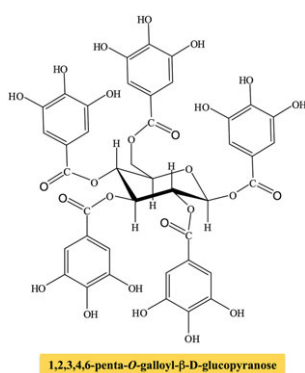
nupharin A, punicalagin, geraniin, eugeniin, davidiin, casuarictin and corilagin), dimeric (e.g. sanguiin), oligomeric (e.g. agrimoniin, nupharin E, nupharin C and hirtellin A) and *C*-glycosidic (e.g. vescalagin, castalagin, casuarinin and stachyurin) molecules. Ellagitannins are formed from gallotannins by intermolecular carbon–carbon coupling between at least two galloyl units, yielding hexahydroxydiphenoyl acid, which in aqueous solution spontaneously lactonizes into ellagic acid (Figure 3). Ellagitannins and ellagic acid have been identified in both fruits as well as in nuts and seeds (Landete, 2011), see Table 1. The oxidative coupling commonly involves C-4 and C-6 (as in the case of eugeniin) and may extend to C-2 and C-3, leading to the formation of casuarictin (Figure 4). Other carbon atoms involved in this reaction are the C-3/C-6, C-2/C-4 or C-1/C-6 pairs. Hexahydroxydiphenoyl acid can undergo esterification not only with glucose but also with hamamelose and terpenoids. Ellagitannins can yield dimers following intermolecular oxidative coupling with other hydrolyzable tannins (as in the case of euphorbins) or high MW oligomers.

Complex tannins. The reaction of gallotannins or ellagitannins with catechin units yields complex tannins. Flavan-3-ols can link at C position of an open-chain glucose. Typical examples of complex tannins are camelliatannins A and B, malabatrin A and acutissimin A (Okuda and Ito, 2011).
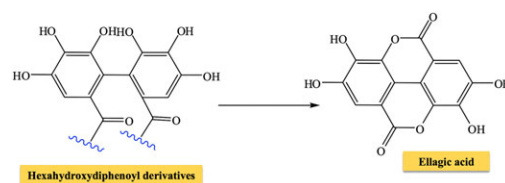

Figure 3

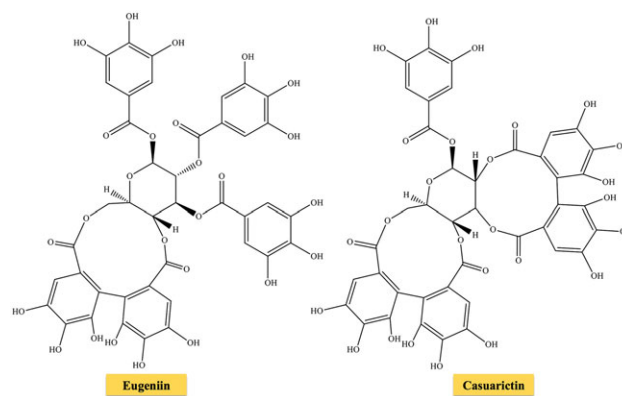Formation of ellagic acid in aqueous solution.

Figure 2

Chemical structure of pentagalloyl-glucose.

Figure 4

Chemical structure of eugeniin and casuarictin.

Distribution and content of proanthocyanidins and hydrolysable tannins in food

Tannins, in particular proanthocyanidins, are one of the most widespread compounds present in food ingested daily. They are commonly found in plants and have been identified in almost all their parts (fruits, leaves, roots, seeds, wood and bark). More than 1000 derivatives have been identified to date, and these compounds continue to be of interest to researchers due to the wide range of applications and the numerous biological processes influenced by their presence. These compounds are found in tea, wine, cereals, cacao and fruits in amounts of up to 184 mg·100 g^{-1}, with epicatechin being the most abundant component, followed by catechin and type B-procyanidin (de Jesus *et al.,* 2012; Lamy *et al.,* 2016). They are also the principal components found in ripe fresh nectarines, peaches and plums (Table 1). Recently, the presence of epicatechin and B-type dimers and trimers of proanthocyanidins has been detected in the pulp of *Annona cherimola* Mill (Barreca *et al.,* 2011). In red wine, the content of catechin, epicatechin, procyanidin B1, B2, B3 and B4 amounts to ~18 mg·100 g^{-1}, more than double that found in white wine (Sanchez-Moreno *et al.*, 2003). Catechin and procyanidin B2 were the two principal components found in some cereals and fruits, as well as in tea and red wine. A complete list of proanthocyanidin content in foodstuffs can be found on the USDA database of Proanthocyanidin Content of Selected Foods (http:// www.nal.usda.gov/fnic/foodcomp/Data/PA/PA.html).

Gallotannins are present only in selected woody and herbaceous dicotyledons. Cereals are sources of hydrolyzable phenols due to phenolic compounds being linked to cell wall components. β-Glucogallin has been reported to be one of the components of mango fruit pulp (Oliveira *et al.,* 2016). Sumac and Chinese gallnuts (or nutgalls) are particularly rich in gallotannins. Sumac (*Rhus coriaria L.*) is a shrub grown in Spain, Italy, Turkey and some Mediterranean Arab countries from which a powder is obtained that has long been used as a tanning agent. The main compounds are D-glucose derivatives esterified with gallic acid, such as penta-, hexa-, hepta-, octa-, nona-, deca-, undeca- and dodeca-galloyl-D-glucose (Lu *et al.,* 2013). Ellagitannins are present in only a few fruits and nuts (especially pecans and walnuts) consumed in the Western diet, and dietary intake (most probably far below 5 mg·day^{-1}) is mainly resulting from the consumption of red fruits (strawberries, raspberries and blackberries or beverages and jams made from these) (Okuda and Ito, 2011), see Table 1. The principal derivatives identified in foods so far are as follows: sanguiin H-6, punicalagin, sanguiin H-5, vescalagin, castalagin, roburin E, casuarictin, pedunculagin, potentillin and lambertianin C. Estimates of the amount of ellagitannins present in food are based on detection of ellagic acid (after hydrolysis of these compounds) followed by HPLC detection, although care is needed during quantification to avoid underestimation deriving from solubility issues. Sanguiin H-6, casuarictin, potentillin and pedunculagin are the predominantly ellagitannins identified in strawberries, raspberries and blackberries (Zorenc *et al.,* 2016). These compounds are mainly contained in the peel and seed, while very low quantities or traces have been detected in the pulp. Pomegranates (fruits, juices and jams) are also a rich source of ellagitannins, mainly punicalagin and ellagic acid (Masci

et al., 2016). Ellagitannins have been found in significant amount (Table 1) also in walnuts and pecan nuts (Landete, 2011), with pedunculagin being the main derivative present in walnuts.

Pharmacokinetic aspects

Aside from the pharmacological effects exerted locally in the gut, tannins have to be bioavailable in order to exert their health effects, and therefore, absorption, bioavailability and metabolism of these compounds must be carefully evaluated. Although the pharmacokinetics of simple phenols have been extensively investigated in animal and human studies (Velderrain-Rodríguez *et al.,* 2014), the same cannot be said for tannins, where results are still scarce and controversial. High MW tannins apparently are not absorbed intact, as demonstrated by Wiese *et al.* (2015), who reported that almost 90% of apple juice-derived procyanidins consumed were retrieved in ileostomy effluent and were able to enter the colon (Wiese *et al.,* 2015). The polymerization rate appears to play a major role in the fate of these compounds and seems to be indirectly related to the absorption of these molecules through the gut barrier and to their metabolism by gut microbiota to the monomers (i.e. catechin and epicathechin) (Zhang *et al.,* 2016). Some *in vivo* and *in vitro* studies have investigated the hydrolysis mechanism of polymeric proanthocyanidins and whether it is possible for the derived oligomeric molecules to be absorbed by the small intestine; often the molecules that appear in biological fluids can be very different from those ingested (Zhang *et al.,* 2016). Even though small quantities of proanthocyanidins monomers and dimers were found in the plasma compartment, methylated and glucuronidated derivatives seem to be the main metabolites. Furthermore, in the colon, the proanthocyanidins are catabolized by the gut microflora into a series of simpler metabolites such as phenyl valerolactone, phenylacetic and phenylpropionic acids (Zhang *et al.,* 2016). However, these results are still controversial.

In vitro studies. Some *in vitro* experiments have suggested, for example that cocoa procyanidins are hydrolyzed into a mixture of epicatechin monomers and dimers under conditions similar to those present in the human stomach. Nevertheless, other studies have demonstrated that no significant depolymerization of cocoa procyanidins occurs in the stomach, thus suggesting a plausible buffer mechanism provided by the food bolus against the acid stomach environment (Ellam and Williamson, 2013; Kim *et al.,* 2014). However, despite these conflicting observations, what is certain is that during digestion, high MW proanthocyanidins can form complexes with proteins, starches and digestive enzymes, resulting in the formation of less digestible complexes and this property is indirectly related to the degree of polymerization. Indeed, small molecules such as dimeric and trimeric proanthocyanidins can be more readily absorbed (Zhang *et al.,* 2016). Absorption of OPCs has been reviewed recently by Ou and Gu, 2014. They reported that procyanidin A-type dimers, trimers and tetramers were slowly transported across Caco-2 cells monolayers (transport ratio of 0.6%, 0.4% and 0.2% respectively), suggesting their possible absorption after

dietary intake. Furthermore, they reported similar permeability coefficients for (+)-catechin and a proanthocyanidin dimer and trimer, while the permeability of proanthocyanidins with high polymerization was found to be approximately 10 times lower.

As described above, most ingested tannins reach the colon. At this level, the gut microbiota play a key role in their metabolism leading to the formation of absorbable or unabsorbable metabolites which remain in the colonic lumen where they can counteract the effects of some pro-oxidant agents (Marín *et al.,* 2015). Proanthocyanidins seem to be extensively metabolized by gut microbiota producing mainly phenylacetic, phenylpropionic and phenylbutyric acids (Marín *et al.,* 2015). The low concentration of ^{14}C-labelled metabolites detected indicates controversial results (Depréz *et al.,* 2000). However, these underestimates may be due to the ability of some polyphenols to bind strongly to various molecules in gut cells and dietary fibres in the colonic lumen.

There is currently no data available regarding the intestinal bacteria responsible for the degradation of proanthocyanidins and their metabolites. Hydrolysable tannins are metabolized to gallic acid, pyrogallol, phloroglucinol and finally to acetate and butyrate via several bacterial enzymes (Marín *et al.,* 2015).

In vivo studies. Despite some evidence to the contrary, the OPCs appear to be hydrolyzed into epicatechin in rat isolated small intestine (Zhang *et al.,* 2016). Nevertheless, orally administered procyanidins in rat plasma and trace amounts of procyanidin dimers and trimers in urine (Li *et al.,* 2013) were detected. These results suggest that proanthocyanidin trimers and dimers can be absorbed *in vivo,* also due to the similarity of the permeability coefficient to that of mannitol, and limited polymer bioavailability in the gut lumen (Ou and Gu, 2014).

Moreover, 16 metabolites, including phenylacetic, phenylpropionic and phenylbutyric acids, have been detected in rat urine after administration of proanthocyanidin isolated from willow tree catechins. The total yields significantly decreased in relation to the polymerization degree of the precursors: catechin monomer > dimer > trimer > polymer (Brenes *et al.,* 2016).

Analysis of luminal content, after administration of grape seed extract (GSE) to Sprague–Dawley rats, showed distinct native and metabolite profiles for each region (caecum and proximal, mid and distal colon) allowing insight to be gained into the distribution and delivery of procyanidins and their microbial metabolites throughout the colon. In particular, procyanidins reached maximum concentrations approximately 3 h postgavage appearing earlier in the more proximal segments, while metabolites reached maximum concentrations from 3 to 18 h post-gavage appearing later in more distal regions (Goodrich *et al.,* 2015). Recently, the systemic absorption and metabolism of dietary procyanidin B4, after oral administration, were evaluated in pigs, demonstrating that this compound is absorbed as an intact molecule and in part excreted in urine. In addition, it was degraded to the monomeric subunits cathechin and epicathechin that were then further metabolized to methylated and glucuronidated conjugates (Bittner *et al.,* 2014).

Human studies. Unlike condensed tannins, few studies on the bioavailability of hydrolysable tannins are currently available. However, the absorption rate of ellagic acid and gallic acid has been extensively documented. Some studies investigating the absorption of main compounds after oral administration of pomegranate juice detected low concentrations of ellagic acid in plasma after 0.5–3 h, while no intact forms of ellagitannins were detected (Nuñez-Sánchez *et al.,* 2014).

The low plasma concentration of free ellagic acid is probably due to its low water solubility and to its ability to complex calcium and magnesium ions in the intestine and thus compromise gut absorption (Serrano *et al.,* 2009). The main ellagic acid derivatives have been found in human plasma and urine (Nuñez-Sánchez *et al.,* 2014). The intestinal absorption of gallic acid in humans is relatively slow (t_{max}, 87 min), as also found in rats (t_{max}, 60 min) (Serrano *et al.,* 2009). Two different speeds of gastric absorption have been suggested for this simple phenol: rapid permeation for the unchanged molecule and slow permeation for the metabolites. The main metabolite of gallic acid observed in humans is 4-*O*-methylgallic acid (Serrano *et al.,* 2009).

A plausible mechanism for ellagitannin degradation by human microbiota, via hydrolysis into ellagic acid and its microbial transformation into urolithin B, has been proposed. A study performed on human volunteers fed with a single dose of ellagitannin-rich dietary sources demonstrated a large inter-individual variation in the metabolite profiles among subjects within each group, suggesting the involvement of variable colonic microbiota (Espín *et al.,* 2013). It is known that tannins are partially bioavailable for absorption in the gastrointestinal tract, small intestine for hydrolyzable tannins and large intestine for proanthocyanidins (Kamiloglu *et al.,* 2016). These data correlate with some *in vitro* antioxidant properties ascribed to these tannins (Brenes *et al.,* 2016).

It may be concluded that absorption through the gut barrier is probably limited to the absorption of tannins with a low degree of polymerization and of their metabolites formed in the colon. In view of this, it is critical to evaluate bioavailability studies in order to gain a better understanding of the effects of these phytochemicals on human health. In addition, the above findings highlight the necessity to choose appropriate biomarkers for the in depth study of the pharmacokinetics of these molecules. For some polyphenols, this has already been achieved (Espín *et al.,* 2013; Stalmach *et al.,* 2014). However, there is a great deal still to be done in this area.

Experimental and clinical pharmacology

The biological properties and health effects of tannins have been extensively reviewed (Coppo and Marchese, 2014; Katiyar, 2015; Salvadó *et al.,* 2015; Verstraeten *et al.,* 2015). Many of the *in vitro* and *in vivo* (human and animal) studies performed suggest that tannin intake may prevent the onset of several chronic diseases, as reported by recent reviews, systematic reviews and meta-analysis (Holt *et al.,* 2012; Blumberg *et al.,* 2013; Wang *et al.,* 2014; Salvadó *et al.,* 2015; Turati *et al.,* 2015). The recognized pharmacological activities have been ascribed to dietary monomeric flavan-3-ol derivatives and to various crude and purified tannins or proanthocyanidin fractions from the whole plant, fruits,

leaves, rhizoma and bark of various medicinal plants (Table 2). Tannins exert their biological effects not only as an unabsorbable (high MW) complex structure with binding properties that may produce local effects in the gastrointestinal tract but also as absorbable tannins (dimers and trimers) with their metabolites producing systemic effects (Jiménez *et al.,* 2014; Nuñez-Sánchez *et al.,* 2014; Ou and Gu, 2014; Velderrain-Rodríguez *et al.,* 2014; Sieniawska, 2015). In addition to their well-documented free radical scavenging and antioxidant activity, tannins seem able to exert antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic and vasodilatory effects (Holt *et al.,* 2012; Blumberg *et al.,* 2013; Nile and Park, 2014; Salvadó *et al.,* 2015; Zhang *et al.,* 2016). An overview of the main pharmacological activities ascribed to these compounds is provided in Table 3 and described in detail below.

Antioxidant and radical scavenging activity. Most activities of the proanthocyanidins and hydrolyzable tannins, including antioxidant and free radical-scavenging capacity, largely depend on their structure; for example, an increase in antiradical effects was observed with an increase in the degree of polymerization (Sieniawska, 2015).

Experimental pharmacology. Proanthocyanidins are known to inhibit lipid peroxidation and lipoxygenases *in vitro*, and several studies have demonstrated their ability to scavenge hydroxyl, superoxide and peroxyl radicals, helping to restore the oxidative balance of the body (Georgiev *et al.,* 2014). In some instances, some procyanidins such as procyanidin B1 and procyanidin B3 have been recognized to be stronger antioxidants than ascorbic acid or α-tocopherol (Iglesias *et al.,* 2012). These results observed *in vitro* have also been confirmed by several *in vivo* studies on animal models (Kancheva and Kasaikina, 2013; Nile and Park, 2014; Sieniawska, 2015; Skrovankova *et al.,* 2015).

Fushimi *et al.* (2015) have investigated the antioxidant effects of unripe and mature persimmon in rats. Results showed that plasma phosphatidylcholine hydroperoxide levels, a biomarker of membrane lipid peroxidation, were significantly lower in the unripe persimmon group than in the control group, while no significant difference was observed between the mature persimmon group and the control group. The authors suggested that soluble tannins, found more in unripe fruits, could contribute to the difference in the antioxidant effect observed (Fushimi *et al.,* 2015). A few years earlier,

Table 2

Main herbal medicines containing tannins and their therapeutic indications. Source: www.ema.europa.eu

Herbal drugs	Active constituents	Herbal preparations	Therapeutic applications
Agrimonia eupatoria L. (herba)	3–11% tannins(mainly proanthocyanidins and small amount of ellagitannins)	Comminuted herbal substance;Tincture (1:5), extraction solvent ethanol 45% (*V/V*); Liquid extract (DER 1:1), extraction solvent ethanol 25% (*V/V*).	Mild diarrhoea;Mild inflammation of the mouth and throat; Minor inflammation of the skin and small superficial wounds.
Hamamelis virginiana L. (dried or fresh leaves)	3–10% tannins (gallotannins and proanthocyanidins)	Tincture prepared from fresh leaves (1:10), extraction solvent ethanol 45%; Liquid extract prepared from fresh leaves (1:1) extraction solvent ethanol 45% V/V; Liquid extract from dried leaves (1:1) extraction solvent ethanol 30% m/m; Liquid extract from dried leaves (1:2) extraction solvent ethanol 60% V/V.	Minor skin inflammation and dryness; Temporary relief of the symptoms associated with haemorrhoids, such as itching, burning sensation or pain; Minor inflammatory conditions of the oral mucosa.
Vaccinium myrtillus L. (ripe, dry fruits)	Proanthocyanidins	Comminuted herbal substance	Mild diarrhoea;Minor inflammations of the oral mucosa.
Quercus robur L. (cortex)	Hydrolyzable tannins (predominant) and proanthocyanidins	Comminuted herbal substance; Powdered herbal substance; Dried extract (DER 5.0–6.5:1) extraction solvent ethanol 50% m/m.	Mild diarrhoea;Minor inflammatory conditions of the oral mucosa and skin; Relief of itching and burning associated with haemorrhoids;
Potentilla erecta (L.) Raeusch.(rhizoma)	5–22% total tannins (15–20% condensed tannins, about 3.5% hydrolyzable tannins)	Comminuted herbal substance;Tincture (1:5), extraction solvent ethanol 70% (V/V); Tincture (1:5), extraction solvent ethanol 45% (V/V); Liquid extract (DER 1:1), extraction solvent ethanol 25% (V/V); Dry extract (DER 3.5–4.5:1), extraction solvent ethanol 60% (V/V)	Mild diarrhoea;Minor inflammations of the oral mucosa;
Rosa centifoglia L. (dried petals)	Proanthocyanidins	Comminuted herbal substance	Mild inflammations of the oral and pharyngeal mucosa;Minor skin inflammation.

Table 3

Main pharmacological activities ascribed to tannins

Properties	Effects	References
Antioxidant and radical scavenging	Scavengers of hydroxyl, superoxide, and peroxyl radicals (Proanthocyanidins); Inhibition of lipid peroxidation and lipoxygenases (Ellagitannins); Stronger antioxidant activity than ascorbic acid or α-tocopherol (Procyanidins B1 and B3); Dose-dependent radical scavenging action of galloylated condensed tannins.	Nile and Park, 2014 Skrovankova et al., 2015 Kancheva and Kasaikina, 2013 Sieniawska, 2015
Anti-cancer	Apoptosis p53-dependent and Bax/Bcl-2 proteins and caspase-3 activation mediated in JB6 C141 cells (Grape seed proanthocyanidins);Reduction of UV-induced oxidative stress-mediated phosphorylation and activation of NF-κB in NHEK cells (Grape seed proanthocyanidins); Inhibition of cellular proliferation and expression of MMP-2 and −9 in DU145 and LNCaP cells (Grape seed proanthocyanidins); Dose-dependent inhibition of cell viability, proliferation and apoptosis in 4 T1, HT29 and LoVo cells (Grape seed proanthocyanidins); G0/G1 phase cell cycle arrest with increase in Cip1/p21 protein levels and decrease of cyclins and cyclin-dependent kinases in MCF-7 and A-427 cells (Grape seed proanthocyanidins); Inhibition of KB, CAL-27, MCF-7, HT-29, HCT116, DU145 and LNCaP cells growth and apoptosis induction (blackberry, black raspberry, blueberry, cranberry, red raspberry and strawberry proanthocyanidins);; Decrease in of UVB-induced immune suppression in mice with induction of IL-12 (Grape seed proanthocyanidins); Apoptosis induction and metastasis inhibition of 4 T1 murine mammary cancer cells (Grape seed proanthocyanidins); Inhibition of induced mammary tumours in Sprague–Dawley rats (Grape seed proanthocyanidins); In vitro and in vivo inhibition of HT29 cell growth (Grape seed proanthocyanidins); Inhibition of prostate tumour growth and progression in TRAMP mice (Grape seed proanthocyanidins);.	Gollucke et al., 2013 Nile and Park, 2014 Skrovankova et al., 2015 Ouédraogo et al., 2011 Sieniawska, 2015 Turati et al., 2015
Antimicrobial	Inhibition of E. coli adherence to uroepithelium (Cranberry proanthocyanidins);Activity against Staphylococcus aureus, Candida albicans and Campylobacter jejuni (Ellagitannins); Antiherpetic activity (hydrolyzable tannins); Inhibition of cytopathic effects of HIV (hydrolyzable tannins); Inhibition of HIV-antigen expression in human lymphotropic virus type I-positive MT-4 cells (hydrolyzable tannins); Activity against Helicobacter pylori (hydrolyzable tannins).	Marín et al., 2015 Nile and Park, 2014 Sieniawska, 2015 de Jesus, 2012
Anti-nutrient	Inhibition of gastrointestinal enzymes (Proanthocyanidins);Reducing capacity on Cr, Fe and Cu with alteration of their absorption (Proanthocyanidins); Modulation of chronic inflammatory bowl diseases (Procyanidin B3).	Nile and Park, 2014 Sieniawska, 2015 Li et al., 2012 Dias et al., 2016
Cardioprotective	Hypocholesterolemic effects (Proanthocyanidins);Preventing myocardial ischaemic injury in adult rats (Proanthocyanidins); Cardioprotection in an experimental model of ischaemia–reperfusion damage (Proanthocyanidins); Anti-atherosclerosi effect due to inhibition of differentiation of monocyte to macrophages (Oligomeric proanthocyanidins isolated from Crataegus oxyacantha L.); Protective effect on doxorubicin-induced cardiac toxicity in rats (Grape-seed proanthocyanidins); Suppression of doxorubicin-induced electrocardiographic and biochemical changes (Grape-seed proanthocyanidins); Protection against acute ischaemic brain damage in rats (Proanthocyanidins); Vascular protective effects (Procyanidin B2); Improved serum antioxidant status and decreased serum C-reactive protein and plasma homocysteine concentrations (Proanthocyanidins); Decreased systolic and diastolic blood pressure (Grape-wine proanthocyanidins).	Blumberg et al., 2013 Wang et al., 2014 Holt et al., 2012 Guler et al., 2011 Mohana et al., 2015 Ammar et al., 2013 Yunoki et al., 2014 Yang et al., 2013
Anti-diabetic and anti-obesity	Inhibition of salivary and pancreatic α-amylase and α-glucosidase activity (Hydrolyzable tannins and proanthocyanidins);Stimulation of glucose uptake and glycogen and lipid synthesis (hydrolyzable tannins and proanthocyanidins);	Gonzalez-Abuin et al., 2015Banihani et al., 2013 Pinent et al., 2012

(continues)

Table 3 (Continued)

Properties	Effects	References
	Reduced plasmatic insulin and improved homeostatic model assessment (HOMA) index (hydrolyzable tannins and proanthocyanidins); Modulation of the active glucagon-like peptide-1 (GLP-1) levels (hydrolyzable tannins and proanthocyanidins); Hypocholesterolemic effect (tannin-rich fibre); Acute effects on postprandial lipaemia, vascular function and blood pressure (Pomegranate ellagitannins); Insulinomimetic properties GLUT4 mediated (Grape seed proanthocyanidins); Insulin anabolic-like and lipostabilizer properties (Grape seed proanthocyanidins); Inhibition of diabetes-induced cataract formation in rats (Cacao proanthocyanidins); Increased antioxidant enzyme activities, induction of metal chelation activity, reduced resistin formation, and inhibition or activation of transcriptional factors such as NF-kB and PPARɣ (Proanthocyanidins).	Gato *et al.,* 2013 Mathew *et al.,* 2012 Kooti *et al.,* 2016 Cascaes *et al.,* 2015 Cock, 2015 Stohs and Ray, 2015 Salvadó *et al.,* 2015

another study had shown that several proanthocyanidin-type dimers, from peanut skins and persimmon pulp, possess high dose-dependent antioxidant potency. B-type dimers showed the highest radical scavenging activity in aqueous systems while A and B-type dimers showed similar antioxidant potency in tissue or lipid systems highlighting the importance of the food matrix effect (Dong *et al.,* 2013).

Protection against lipid peroxidation has also been observed in several tannin-rich plants. For example, the ethanolic extract of *Nigella sativa* L. was found to counteract *in vivo* Fe(II)-induced lipid peroxidation (Hassan *et al.,* 2016). Also, *Centella asiatica* L. and its fractions were found to reduce lipid peroxidation induced by quinolinic acid and sodium nitroprusside in the rat brain (Marques *et al.,* 2015).

Free radical-scavenging activity of OPCs from *Rhodiola rosea* L. has been evaluated in mice serum, heart, liver and brain tissues. These compounds enhanced SOD and GSH peroxidase (GPx) activity by reducing malondialdehyde (MDA) content (Zhou *et al.,* 2014). Another interesting and recent study has evaluated the protective effect of grape seed proanthocyanidins (GSPs) on cadmium-induced renal toxicity (Nazima *et al.,* 2015). Proanthocyanidins were also found able to improve lead-induced cognitive impairments by blocking endoplasmic reticulum stress and NF -κB-mediated inflammatory pathways in rats (Liu *et al.,* 2014).

A meta-analysis of experimental studies regarding the efficacy of proanthocyanidins against oxidative damage has recently been performed (Li *et al.,* 2015a). Compared with the control group, proanthocyanidins significantly improved total antioxidative capacity, SOD, GSH, GPx and catalase (CAT) and reduced the MDA levels. Proanthocyanidins therefore effectively antagonize oxidative damage and enhance antioxidant capacity, but the antagonistic effect may be related to intervention time, intervention method and biological sample from which the indexes are estimated. The authors observed, in fact, significant differences in the effects of proanthocyanidins in relation to themode of administration (gavage vs. feeding) and dependent on the biological matrix analysed (Li *et al.,* 2015a).

Clinical pharmacology. At present, there is only limited clinical evidence available about the antioxidant properties of hydrolyzable tannins and proanthocyanidins. Three pilot studies on human volunteers have recently been carried out using a French oak wood (*Quercus robur* L.) extract rich in roburin (a dimeric tannin), known with the brand name of Robuvit®. The first examined the pharmacokinetics and effects at transcriptome level of this formulation in healthy volunteers and showed that Robuvit® metabolites affected ribosomes, cell cycle and spliceosome pathways (Natella *et al.,* 2014). The second study, which analysed the effect of 1 month intake of Robuvit® (300 mg·day^{-1}) on oxidative stress markers in the plasma of healthy volunteers, demonstrated a decrease of the advanced oxidation protein products and lipid peroxides, an increased SOD and CAT activity and total antioxidant capacity (Horvathova *et al.,* 2014). The third study, which was the most recent, showed Robuvit® to have no significant influence on GSH levels and paraoxonase (an antioxidant enzyme) activity, although homocysteine and cysteine levels decreased significantly (Deáková *et al.,* 2015).

Finally, a double-blind crossover trial was performed on 18 volunteers to evaluate the effects of procyanidin-rich chocolate on fecal free radical production and antioxidant activity. Subjects were fed a low-polyphenol diet and chocolate for two 4 week periods separated by a 4 week washout period. The authors observed a decreased free radical production in fecal water with respect to control group, suggesting that chocolate proanthocyanidins may be effective in rebalancing redox status (Sieniawska, 2015).

Anti-cancer properties. Many studies, carried out to highlight the mechanisms of action of proanthocyanidins, have identified several molecular targets potentially useful for the prevention and treatment of cancer. It should be stated, however, that knowledge in this area is still at an embryonic stage, and further studies are needed to better clarify the anti-cancer properties of these compounds.

Experimental pharmacology. Proanthocyanidins have been extensively studied on a wide range of cancer cells. GSPs induced p53-dependent apoptosis on JB6 C141 mouse skin epidermal cells, through involvement of Bax/Bcl-2 proteins and caspase-3 activation (Gollucke *et al.,* 2013).

Furthermore, inhibition of the phosphorylation of proteins belonging to the MAPK family and activation of NF-κB and its related genes were observed in normal human epidermal keratinocytes (Gollucke *et al.,* 2013). The same molecular mechanisms were found to be involved in the inhibition of cell growth and in the down-regulation of MMP-2 and -9 in both androgen-insensitive (DU145) and in androgen-sensitive human prostate carcinoma cells (LNCaP) (Gollucke *et al.,* 2013) on which GSPs also seem able to regulate androgen receptor-mediated transcription through potent anti-histone acetyltransferase activity (Park *et al.,* 2011). The down-regulation of these MMPs , involved in tumour development and metastasis, was also observed in pancreatic carcinoma cells (Chung *et al.,* 2012). The inhibition of cell viability and proliferation and the pro-apoptotic activity of GSPs were also observed on *mouse mammary tumour* 4T1 and colorectal cancer HT29 and LoVo cell lines (Sieniawska, 2015). GSPs interfered with normal regulation of cell-cycle progression inducing an up-regulation of Cip1/p21 and Kip1/p27 protein levels together with a down-regulation of cyclins (D1, D2 and E) and cyclin-dependent kinases (CDK2, CDK4 and CDK6) leading to Go/G1 phase arrest (Prasad and Katiyar, 2014). Concentration-dependent effects of GSPs were observed on breast and lung cancer and gastric adenocarcinoma cells (Gollucke *et al.,* 2013).

Besides GSPs, grapes and pine bark procyanidin-rich fractions (with the highest percentage of galloylation and mean degree of polymerization) also efficiently inhibit cell proliferation, arresting the cell cycle in G2 phase and inducing apoptosis, as observed in HT29 human colorectal cancer cells (Ouédraogo *et al.,* 2011). *In vitro* anti-proliferative and pro-apoptotic effects were also observed with several berry proanthocyanidin-rich extracts on human oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT116) and prostate (LNCaP) cancer cells (Nile and Park, 2014; Skrovankova *et al.,* 2015).

The *in vitro* anti-cancer properties of proanthocyanidins have been confirmed by several animal studies. Dietary feeding of GSPs was found to inhibit UVB-induced photocarcinogenesis in SKH-1 hairless mice. This event seems to be correlated with the ability of these compounds to decrease UVB-induced immune suppression in mice, by enhancing the production of IL-12 and reducing the expression of IL-10 (Gollucke *et al.,* 2013; Sieniawska, 2015). A study performed in immunocompetent Balb/c mice fed with GSPs showed tumour-growth inhibition with an increase in the survival period and inhibition of metastatic status (Gollucke *et al.,* 2013). Similar results were observed in other *in vivo* cancer models (Gollucke *et al.,* 2013). Moreover, it has been demonstrated that GSPs inhibit the spontaneous development of prostate cancer in male TRAMP mice (Sieniawska, 2015).

Clinical pharmacology.   Although a number of experimental studies have been published, few clinical trials on the anti-cancer properties of proanthocyanidins are available, to date.

A double-blind, placebo-controlled, randomized phase II trial was planned to elucidate the effects of supplementation with GSP extract (300 mg·day^{-1} for 6 months) in patients affected by radiation-induced breast induration. At 12 months post-randomization, ≥50% reduction in surface area (cm^2) of breast induration in 29.5% of GSPs extract treated patients was recorded, while no significant difference between treatment and control groups in terms of external assessments of tissue hardness, breast appearance or patient self-assessments of breast hardness, pain or tenderness was recorded (Sieniawska, 2015).

Proanthocyanidins have been also evaluated for their potential to attenuate the adverse effects of cancer radiotherapy. A randomized double blind, placebo-controlled, pilot study was conducted using standardized cranberry capsules, containing 72 mg of proanthocyanidins, for the prevention and treatment of radiation cystitis in prostate cancer patients. The authors observed a statistically significant decrease in cystitis incidence in treated patients (65%) compared with control group (90%); severe cystitis was reported in 30% of treated patients and 45% in the control group. Moreover, the incidence of pain and burning was significantly lower in treated group. The results demonstrate that patients who receive radiation therapy for prostate cancer, particularly those on low hydration regimens or with baseline urinary symptoms, may benefit from cranberry supplementation (Hamilton *et al.,* 2015).

Antimicrobial activity. The antimicrobial properties of proanthocyanidins and HTs present in many medicinal plants and foods are well documented. These phytochemicals seem to affect bacterial growth via several mechanisms such as inhibition of extracellular enzymes, deprivation of microbial essential substrates (for example by complexing metal ions), disintegration of bacterial outer membrane with cytoplasm leak or by direct action on microbial metabolism. Furthermore, these compounds can interfere with microbial cell wall polypeptides reacting with sulfhydryl groups, often leading to loss of function of the membrane proteins (Marín *et al.,* 2015).

Experimental pharmacology.   The anti-adhesion properties of berry fruits and derived products have long been known. Cranberry juice, for example, inhibits *E. coli* adherence to uroepithelium. However, the molecular structure of proanthocyanidins seems to play a major role in conferring bacterial anti-adhesion activity to each matrix. In fact, cranberry proanthocyanidins, characterized by a series of catechin oligomers with one or more A-type linkages – and therefore structurally quite different with respect to proanthocyanidins from other sources like grape and apple– have been observed to be the only ones able to elicit bacterial anti-adhesion activity in humans (Micali *et al.,* 2014). These results have been confirmed by several animal studies, which suggest that cranberry proanthocyanidins and/or their metabolites are able to inhibit bacterial adhesion to uroepithelium (Micali *et al.,* 2014).

Cloudberry, raspberry and strawberry extracts, rich in ellagitannins, were found to be the best inhibitors against *Campylobacter jejuni* and *Candida albicans* (Lipińska *et al.,* 2014). Bilberry and blueberry extracts showed bacteriostatic activity on Gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus fecalis*) and Gram-negative bacteria (*Citrobacterfreundii*, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella enteric* ser. *Typhimurium*)

(Nile and Park, 2014). The bacteria most sensitive to berry extracts were *Helicobacter pylori* and *Bacillus cereus*, while yeasts were found to be resistant to the extracts (Lipińska *et al.,* 2014).

Several ellagitannin-rich plants have shown antibacterial properties. For example, *Pteleopsis hylodendron* Mildbr. extracts, containing mainly ellagic acid, were active against *Klebsiella pneumoniae*, *B. cereus*, *E. coli* and *Salmonella typhi* (Marín *et al.,* 2015). Pomegranate peel extract has also been observed to be active against *S. aureus*, *S. typhi*, *L. monocytogenes* and *E. coli.* Ellagic acid was found able to inhibit biofilm formation by *S. aureus*, including themethicillin-resistant strains, and *E. coli,* as well as by the fungal pathogen *C. albicans* (Bakkiyaraj *et al.,* 2013). Antifungal properties against *C. albicans, C. neoformans* and *Aspergillus fumigates* have been shown by this compound as well as by punicalagin, punicalin and gallagic acids (Marín *et al.,* 2015).

Antimalarial activity against chloroquine and mefloquine-resistant *Plasmodium falciparum* has been reported for ellagic acid, which showed synergy when combined with conventional antimalarial drugs (Marín *et al.,* 2015).

Antiviral activity has also been described and seems to be strictly related to the tannin structure. The most active tannins appear to be the most cytotoxic. Several studies have suggested that tannins may interfere with *Herpes simplex* virus absorption as well as the absorption of HIV and its cytopathic effects (Lipińska *et al.,* 2014; Ekambaram *et al.,* 2016). This mechanism seems to be related to the capacity of tannins to bind the viral envelope components. Furthermore, ellagitannins and several proanthocyanidins have been demonstrated to be potent reverse transcriptase inhibitors (Ekambaram *et al.,* 2016). One hydrolyzable tannin, ellagic acid, also shows specific antiviral activity against hepatitis B virus (HBV) by inhibiting HBeAg secretion in HBV-infected cells (Marín *et al.,* 2015).

Clinical pharmacology. There are several clinical studies on dietary supplements containing ellagitannins or proanthocyanidins and their antimicrobial properties and the most recent are discussed below.

A randomized, double-blind, placebo-controlled, acute study was performed to evaluate the *E. coli* anti-adhesion activity following administration of several cranberry extracts, containing a standardized level of 36 mg proanthocyanidins, in healthy subjects. The authors observed, for all formulations studied, a significantly anti-adhesion effect after intake of a single dose of cranberry extracts compared with the control group (Howell *et al.,* 2015).

Another randomized, placebo-controlled study was carried out in order to clarify how cranberry fruit powder (500-mg·per day for 6 months), with a standardized 0.56% proanthocyanidin content, could prevent recurrent urinary tract infection (UTI) in 182 women with two or more UTI episodes in the previous year. Results showed significantly fewer UTIs in the cranberry group with respect to the control group (10.8% vs. 25.8% respectively). The treated group also showed a much longer latency period before developing a new UTI episode than the placebo group (Vostalova *et al.,* 2015). It should be mentioned, however, that the ability of cranberry to prevent UTIs has been recently questioned (Jepson *et al.,* 2013; Izzo *et al.,* 2016).

The health benefits of pomegranate consumption are attributed, as reported above, to ellagitannins and their metabolite content. In a recent study, performed on 20 healthy volunteers consuming 1000 mg·day^{-1} of pomegranate extract for 4 weeks, three different behaviours – based on urinary and fecal content of the pomegranate metabolite urolithin A – were observed: 1) in nine subjects no baseline urolithin A was present but its formation was induced by pomegranate extract consumption; 2) in five subjects baseline urolithin A formation was present and enhanced by pomegranate extract consumption; and 3) in six subjects there was no baseline urolithin A production and none was induced. Results showed an increase of *Actinobacteria* and *Verrucomicrobia* phyla and a decrease of *Firmicutes* in urolithin A producers with respect to baseline values. After 4 weeks, *Enterobacter*, *Escherichia*, *Lactobacillus, Prevotella*, *Serratia* and *Veillonella* genders also increased, in urolithin A producers compared with baseline values, while *Collinsella* decreased significantly. On this basis, the authors suggested that health benefits may be induced by pomegranate extract consumption due to changes in the microbiota (Li *et al.,* 2015b).

Anti-nutrient activity. Tannins, known to be astringent compounds due to their ability to complex and precipitate proteins, particularly proline-rich proteins, are present in the human saliva through hydrophilic and hydrophobic interactions. Recently, a study was performed to elucidate the interaction between procyanidin B3, a common food tannin, and different wheat-derived proline-rich peptide fractions, responsible for the onset of celiac disease (CD). This study identified several soluble B3-peptide complexes containing immunoreactive peptides, of different sizes and diversity in CD epitopes, demonstrating the potential beneficial effects of proanthocyanidins as a nutritional approach in the modulation of gut diseases (Dias *et al.,* 2016).

The complexing ability of proanthocyanidins can also inhibit enzymes (pectinase, amylase, lipase, protease and β-galactosidase) and may interfere with absorption of other compounds like proteins and carbohydrates. Several *in vitro* studies have supported this claim. However, the *in vivo* studies have not confirmed it, except at high doses, probably due to adaptation mechanisms such as induction of bile acids secretion in the gut and increase of proline-rich salivary proteins (Brandão *et al.,* 2014).

The limited absorption through the gut barrier of proanthocyanidins with a high degree of polymerization and the relatively weak affinity of those with a low polymerization degree, for proteins, have led to the hypothesis that non-specific binding of proanthocyanidins to proteins is involved in the biological properties exerted by these compounds in inner tissues (Sieniawska, 2015). On the contrary, greater affinity to the shortest human saliva peptides has been observed (procyanidins C2 > B1 > B3) (Cala *et al.,* 2012). Tannins have also been found to possess reductive capacity for some metals, for example Cr, Fe and Cu, thereby reducing their absorption (Sieniawska, 2015; Li *et al.,* 2012).

No data are available from human studies about tannins and their anti-nutritional effects.

Cardioprotective properties. The links between proanthocyanidin intake and cardioprotective properties have been extensively reviewed (Kay *et al.,* 2012; Wang *et al.,* 2014; Sieniawska, 2015; Bladé *et al.,* 2016). A number of *in vitro* and animal studies have recently been performed to elucidate the role of proanthocyanidins as promising molecules that could prevent the development of several coronary syndromes by inhibiting the atherogenic process and balancing blood pressure and lipid homeostasis (Hort *et al.,* 2012; Pons *et al.,* 2014; Quifer-Rada *et al.,* 2016).

Recently, other mechanisms of action have been proposed. For example, an *in vitro* and *in vivo* study showed inhibition of monocyte to macrophages differentiation in atherosclerosis, by OPC isolated from *Crataegus oxyacantha* L. In summary, the OPCs decreased vascular cell adhesion protein 1 andchemokine CCL2 levels, down-regulating the inflammatory pathway and macrophage markers, MMP 2 and 9 and PPARγ. These results highlight the potential role of these phytochemicals in the initial stages of atherosclerosis development and in overt disease. dependent on macrophage function (Mohana *et al.,* 2015). Another animal study evaluated the cardio-protective effect of GSPs on doxorubicin-induced cardiac toxicity in rats. Administration of proanthocyanidins significantly suppressed doxorubicin-induced electrocardiographic changes and normalized the aconitine dose producing ventricular tachycardia. Furthermore, proanthocyanidins significantly suppressed the biochemical changes induced by doxorubicin on creatine kinase-myocardial band, LDH, MDA, SOD, CAT and reduced GSH (Ammar *et al.*, 2013).

GSPs have also been found to possess antioxidant, anti-inflammatory and antiapoptotic effects through which they are able to protect the liver against ischaemia/reperfusion injury by attenuating endoplasmic reticulum stress (Xu *et al.*, 2015). In addition, there is evidence that a proanthocyanidin-rich diet protects against acute ischaemic brain damage in rats, improving motor function, reducing cerebral infarction volume and decreasing both peroxidative markers such as 4-hydroxynonenal, advanced glycation end products, 8-hydroxy-2-deoxyguanosine as well as inflammatory markers such as CCL2, ionized calcium-binding adapter molecule-1 and TNF-α (Yunoki *et al.,* 2014).

Finally, another recent study investigating whether procyanidin B2 inhibits NLRP3 inflammasome activation in endothelial cells showed a significant down-regulation of NLRP3 inflammasome in HUVECs followed by an inhibition of caspase-1 activation and IL-1β secretion in response to LPS. In addition, procyanidin B2 reduced LPS-induced formation of ROS and down-regulated the transcriptional activity of activator protein-1 (Yang *et al.,* 2014).

Clinical pharmacology. A cross-sectional study has recently demonstrated the potential role of dietary antioxidants, including proanthocyanidins, in the prevention of cardiovascular diseases because they are able to improve serum antioxidant status and decrease serum C-reactive protein and plasma homocysteine concentrations (Yang *et al.,* 2013).

In a double-blind, placebo-controlled crossover study, the effect of two grape extracts (grape-red wine and grape alone) on blood pressure and vascular function was assessed in mildly hypertensive subjects. Results showed that only the grape wine extract, rich in catechins and proanthocyanidins, was effective in decreasing the 24-hour ambulatory systolic and diastolic blood pressure and the plasma concentration of the vasoconstrictor endothelin-1, demonstrating that catechins and procyanidin-rich food products may help to maintain physiological blood pressure and contribute to preventing the onset of heart disease (Draijer *et al.,* 2015).

Also, a randomized, double-blind, placebo-controlled study performed in women affected by menopausal symptoms supplemented with GSPs tablets showed an improvement of physical and psychological symptoms (Terauchi *et al.,* 2014).

In conclusion, while several *in vitro* and animal studies are available, clinical trials are sparse and have yielded controversial results. Further human studies are needed to confirm the preliminary findings obtained so far.

Metabolic disorders. A number of reviews and systematic reviews have recently been published about the *in vitro* and animal studies available, elucidating the role of proanthocyanidin-rich plant extract in the treatment of diabetes, diabetic complications and other metabolic disorders like obesity (Bertoia *et al.,* 2015; Gonzalez-Abuin *et al.,* 2015; Akaberi and Hosseinzadeh, 2016; Bladé *et al.,* 2016). They highlight the main pharmacological mechanisms by which tannins may act on metabolic disorders.

Experimental pharmacology. Several molecular mechanisms and molecular targets have been proposed to explain the role of proanthocyanidins and hydrolyzable tannins in metabolic disorders. These phytochemicals have been found able to: (i) stimulate glucose uptake and glycogen and lipid synthesis by activation of insulin receptors, PKB, p44/42 and p38 MAPKs signalling pathways and translocation of glucose transporter type 4 (GLUT4) to the plasma membrane; (ii) exert insulin-like effect on insulin-sensitive tissues; (iii) delay the onset of insulin-dependent diabetes mellitus by regulating the antioxidant status of pancreatic beta cells; (iv) reduce plasmatic insulin and improve the homeostatic model assessment (HOMA) index by down-regulation of PPARγ2, GLUT4 and insulin receptor substrate 1 in mesenteric white adipose tissue (WAT); (v) modulate active glucagon-like peptide-1 levels; (vi) inhibit intestinal enzymes like α-amylase (proanthocyanidins) and α-glucosidase (hydrolyzable tannins); (vii) inhibit both salivary and pancreatic amylase; and (viii) induce triglyceride turnover (Banihani *et al.,* 2013; Cascaes *et al.,* 2015; Cock, 2015; Gonzalez-Abuin *et al.,* 2015; Salvadó *et al.,* 2015; Stohs and Ray, 2015; Kooti *et al.,* 2016).

Clinical pharmacology. Despite the wealth of experimental evidence available regarding tannins and metabolic disorders, human studies are still scarce, and findings to date are controversial.

A randomized, double-blind, placebo-controlled trial, which investigated the hypocholesterolemic effects of persimmon fruit tannin-rich fibre, showed a significant decrease of total plasma cholesterol levels as well as of LDL cholesterol levels with no changes in plasma HDL cholesterol or plasma triglyceride levels were observed (Gato *et al.,* 2013).

Another randomized, controlled crossover trial was conducted on 19 young, healthy men with an energy drink (containing ellagitannin-rich pomegranate extract). Results showed no significant differences in terms of capillary plasma triacylglycerol (TAG) between treated groups and control group while systolic blood pressure was found to be higher in treated groups than the control group. No appreciable differences were observed for reflection and stiffness indices or diastolic blood pressure (Mathew *et al.,* 2012).

Safety and toxicity

When discussing t safety, it is important to make a distinction between biofunctional constituents in foods and the same molecules as purified or semi-purified compounds, as found

Table 4

Current available list of completed clinical trials provided by U.S. National Institutes of Health (www.clinicaltrials.gov)

Number ID	Title	Subjects enrolled
NCT01688154	Ability of Grape Seed Proanthocyanidins to Reduce Postprandial Triglycerides in Humans	Both (20–40 years)
NCT00742287	Cardiovascular Effects of Oligomeric Procyanidins (OPCs) in Smokers	Male (30–60 years)
NCT01483508	Absorption and Metabolism of Dietary Cocoa Procyanidins in Humans	Both (18–70 years)
NCT01687114	Urinary Proanthocyanidin-A2 as a Biomarker of Compliance to Intake of Cranberry Products	Both (20–40 years)
NCT02515929	Prospective Double-Blind Randomized Controlled Clinical Trial in the Gingivitis Prevention With OPCs	Both (18–50 years)
NCT01707615	Beneficial Effects of Grape Seed Proanthocyanidin Extrat on Progression of Atherosclerotic Plaques in Clinical Use	Both (43–75 years)
NCT02408289	The Randomized Controlled Cocoa-Appetite Trial	Male (18–35 years)
NCT02039648	The Influence of Rumex Acetosa L on the Intraoral Colonization With Porphyromonas Gingivalis	Both (>18 years)
NCT01219595	Cranberry Proanthocyanidins for Modification of Intestinal E. Coli Flora and Prevention of Urinary Tract Infections in UTI-Susceptible Women	Female (18–65 years)
NCT01669317	Mechanisms Underlying the Sleep Promoting Effect of Cherry Juice Standardized to Its Proanthocyanidin Content	Both (>65 years)
NCT01099150	Dark Chocolate and Platelet Function in Humans	Both (18–70 years)
NCT00100893	IH636 Grape Seed Extract in Preventing Breast Cancer in Postmenopausal Women at Risk of Developing Breast Cancer	Female (40–75 years)
NCT02087735	Measurement of Urinary Catabolites of PACs as Biomarkers of Consumption of Cranberry Extracts	Female (18–40 years)
NCT01969994	Absorption, Metabolism, and Excretion of (−)-[2-14C]Epicatechin in Humans	Male (18–50 years)
NCT01847053	Bioavailability Study of Cinnamon in Healthy Subjects	Male (18–40 years)
NCT01010841	Trial of Two Dietary Programs on Cardiometabolic Risk Factors in Subjects With Metabolic Syndrome	Female (20–75 years)
NCT01289860	Investigating the Acute Effects of Flavonoids in Blueberries on Cognitive Function.	Both (18–75 years)
NCT00568152	Effect of Apple Flavanols on Risk of Cardiovascular Disease	Both (19–64 years)
NCT02063477	Effect of Oligopin® on Blood Pressure.	Both (>18 years)
NCT00713167	The Efficacy of Red Grape Seed Extract on Lipid Profile and Oxidized Low-Density Lipoprotein (OX-LDL)	Both (21–64 years)
NCT01398150	Cranberry Enhances Human Immune Function and Reduces Illness	Both (18–50 years)
NCT00740077	Bioavailability of Flavonoids and Phenolic Acids From Cranberry Juice Cocktail in Healthy Older Adults	Both (50–70 years)
NCT01691430	A Trial of Cranberry Capsules for Urinary Tract Infection Prevention in Nursing Home Residents	Female (>65 years)
NCT00318019	Effect of OPC Factor on Energy Levels	Both (45–65 years)
NCT02333461	Evaluation of Botanicals for Mechanisms Related to Appetite and Fat Metabolism	Both (18–70 years)
NCT01346774	Preventing Urinary Tract Infection Post-Surgery	Female (>18 years)
NCT01681394	Effect of the Administration of a Polyphenol-rich Cocoa Extract on Peripheral Blood Mononuclear Cells Gene Expression	Male (18–40 years)

in dietary supplements. The latter, in fact, contain high amounts of bioactive compounds, and their contribution to overall dietary intake of tannins needs to be taken into account. The dangers of diets containing very high levels of these compounds together with food-based polyphenol enrichment and supplements with purified agents or mixtures have not been studied in detail (Margină *et al.,* 2015). This fact makes polyphenol consumption potentially problematic in terms of alterations in bioavailability and the consequent possible occurrence of side effects due to pharmacokinetic interactions. In theory, the risk related to dietary ingestion of these substances is very low due to their poor bioavailability. Moreover, there are encouraging results from some epidemiological studies reporting a dietary intake of more than 1 g·day^{-1} (i.e. related to eating habits) (Kumari and Jain, 2012) to be associated with a reduced onset of many chronic diseases (Sieniawska, 2015). However, further studies on the potential adverse events that might be associated with high intakes of these constituents are needed.

Animal studies. Tannin toxicity is based essentially on three mechanisms: (i) microbial enzyme inhibition (i.e. cellulases, pectinases, xylanases, GPx, laccase and glycosyltransferase) and substrates for decreased microbial growth (polysaccharides such as pectins, hemicelulose or polygalacturonic acid and less frequently peptides or aminoacids probably due to the lower affinity of tannins for these small molecules with respect to polymers), closely related to tannin-protein interactions; (ii) action on membranes (inactivation of membrane-bound proteins; inhibition of oxidative phosphorylation acting as ionophores; inhibition of electron transport system); and (iii) chelation of metal ions (e.g. iron and zinc) (Guil-Guerrero *et al.,* 2016).

Toxicology studies on tannic acid have so far been limited to the evaluation of genotoxicity, short-term repeat-dose toxicity and carcinogenicity. For example, a subchronic toxicity study, in female rats, demonstrated that a dietary intake of 2.5% grape seed or grape skin extract produced a no-observed-adverse effect level (NOAEL) (Serrano *et al.,* 2016). Subchronic oral toxicity of GSE was also examined in another study on rats; the NOAEL of dietary GSE was found equal to 1.4 g·kg^{-1} body weight·day^{-1} in males and 1.5 g·kg^{-1} body weight·day^{-1} in females (Fiume *et al.,* 2014). Furthermore, the LD$_{50}$, after grape seed and skin extract ingestion in rats, was found to be higher than 5 g·kg^{-1} body weight.

Although numerous studies on the mutagenicity/ genotoxicity are available (Kaur *et al.,* 2000; Lehmann *et al.,* 2000; Fuentes *et al.,* 2006; de Rezende *et al.,* 2009; Lluís *et al.,* 2011), many of them are not validated for evaluating genotoxic risk or do not conform with modern protocols as outlined in the current OECD methodological guidelines, so further investigations in this field are highly recommended. Furthermore, no studies on reproductive toxicology are available. What can be said is that hydrolysable tannins are poorly absorbed *per se* by the gut lumen because they are extensively degraded in the gastrointestinal tract. Furthermore, their metabolites in target species, experimental animals and humans are very similar and appear to be efficiently excreted, so the expected lack of residues in foods of animal origin suggests that it is unlikely that consumers will receive an appreciable amount of the parent compound or its metabolites such as to exert a potentially toxic effect (EFSA, 2014).

Human studies. Although many *in vitro* and *in vivo* studies on tannins, and in particular proanthocyanidins, have provided important information with respect to the potential health effects of dietary intake of these compounds, to date, only 35 clinical trials are available, of which 27 are complete, five ongoing and three with unknown status (www.clinicaltrials. gov) (Table 4). Very few of these studies have been performed on potentially more susceptible subjects such as postmenopausal women, and there are even fewer or no studies at all relating to other vulnerable groups, including pregnant and lactating women (Smeriglio *et al.,* 2014b), children and the elderly. Information regarding potential adverse events cannot be more comprehensively assessed until this data becomes available.

Conclusion

Tannins are bioactive compounds that are widely found in nature, and commonly present in edible and medicinal plants. Nowadays, most of the interest in this field derives from the possible implications of tannin intake for disease prevention, and, to this end, significant advances in understanding the biological properties of these bioactive compounds have been made. Current epidemiological and clinical data have shown a very preliminary correlation between tannin intake and health benefits in humans, although further studies are necessary to better investigate the effect of tannins in particular categories of the population. However, there are still numerous issues to be addressed. One of these is the lack of scientific rigour, especially as far as experimental design is concerned. The common use of non-standardized food or plant extracts generates results that are often controversial and difficult to interpret. Furthermore, studies on the pharmacological targets and the mechanisms of action by which tannins exert their biological effects *in vivo* are still scarce. Further studies on the pharmacokinetic, safety and toxicological features should be performed to gain a better understanding of bioavailability, metabolism, tissue distribution and behaviour of these complex molecules, also in relation to other bioactive compounds in order to identify possible interactions and adverse effects that may arise from the co-administration or by use of dietary supplements. In some cases, polyphenol intake can easily reach very high levels normally not encountered in the typical diet. Given the peculiar pharmacokinetics of tannins, there are other aspects that should not be overlooked, that is the food matrix constituents which could exert their effects in the intestinal lumen. Also, the genetic aspects of individuals that could affect gut cell uptake. Finally, individual variations in microbiota can affect the metabolism and, thus, the ultimate health effect in some cases. In our opinion, all these aspects should be taken into account, and an appropriate risk–benefit assessment should be made before making claims about the health effects in humans.

Conflict of interest

The authors declare no conflicts of interest.

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