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Proanthocyanidins in grape seeds: An updated review of their health benefits and potential uses in the food industry

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ABSTRACT

Grape seeds are rich sources of proanthocyanidins, which comprise polyhydroxyflavan oligomers or polymers. The beneficial health properties of grape seed proanthocyanidins are attributed to their conjugated and colonic metabolites. There is potential for a two-way relationship between the gut microbiota and grape seed proanthocyanidin. In particular, numerous *in vitro* and *in vivo* studies have demonstrated that grape seed proanthocyanidins appear to exert pharmacological effects. These include anti-oxidant, anti-microbial, anti-obesity, anti-diabetic, anti-neurodegenerative, anti-osteoarthritis, anti-cancer, and cardio- and eye-protective properties. In this review, it is aimed to summarize the current literature regarding grape seed proanthocyanidins, focusing on the recently proposed mechanisms of action from clinical trials considered to underlie pharmacological and disease-preventing properties, along with their bioavailability, toxicology, and safety with regard to potential utilization in the food industry.

1. Introduction

Grapes (*Vitis* spp.) are among the world's most commonly manufactured fruit crops. Approximately 75 million tonnes are produced annually, of which 41% is grown in Europe, 29% in Asia, and 21% in the United States. They are harvested in temperate areas, where warm summers and rather mild winters comprise typical climatic patterns (FAO-OIV, 2016). Approximately 50% of grapes are used to produce wine, one third are used as fresh fruit, and the rest are refined to produce foods such as jam, juice, grape seed extract, jelly, grape seed oil, dried grapes (raisins), and vinegar (FAO-OIV, 2016). Grapes are among the fruits richest in carbohydrates (17 g/100 g), have a high caloric content (65 kcal/100 g), and a relatively low glycemic index. In addition to being an exceptional source of manganese and potassium, grapes are also a fine source of vitamins B_6, C, thiamine, are among the richest sources of polyphenols.

Polyphenols constitute the most prevalent class of subordinate metabolites. They exist in almost every part of the plant, with over 8000 phenolic formations currently acknowledged (Pietta, Minoggio, & Bramati, 2003). All tannins are polyphenols, although not all polyphenols are tannins, which comprise a divergent group of polyphenolic compounds that dissolve in water. Based on their origin, tannin chemistry differs markedly, containing as many as 20 hydroxyl groups and having a high molecular weight of 500–3000 Da (Khanbabaee & van Ree, 2001). The capability of tannins to confine proteins underlies, in part, their protective features (Constabel, Yoshida, & Walker, 2014) along with their nutritional benefits (Li & Hagerman, 2013). Tannins are either shiny or loose, light yellow or white powders, with a distinctive smell and astringent taste (Smeriglio, Barreca, Bellocco, & Trombetta, 2017). Low molecular weight proanthocyanidins are present in very low concentrations. Astringency and tanning properties are associated with the higher molecular weight proanthocyanidins. Concise tannins, also termed catechin tannins or proanthocyanidins, consist of polymers or oligomers of flavan-3-ol units, and are not readily hydrolysed (Serrano, Puupponen-Pimiä, Dauer, Aura, & Saura-Calixto, 2009).

Overall, these compounds have become increasingly popular, as confirmed by the growing number of functional foods combining them in various formulations and claiming beneficial health effects including antioxidant, anti-inflammatory, anti-allergic, anti-cancer, immune-stimulating, anti-viral, cardio-protective, and antithrombotic features (Martinez-Micaelo, González-Abuín, Ardèvol, Pinent, & Blay, 2012; Pinent et al., 2016; Salvadó, Casanova, Fernández-Iglesias, Arola, & Bladé, 2015). The present review focuses on proanthocyanidins in grape seed, examining their history, chemical structure, occurrence, metabolism and bioavailability, industrial applications, and recent findings regarding their mechanisms and protective effects against diverse diseases.

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2. Historical perspective

The grape is considered to be native to a region near the Caspian Sea in Southwestern Asia. Wine cultivars were brought to Rome, Southern France, and Greece by the Phoenicians, with the Romans in turn spreading the grape throughout Europe. In the 1700 s, Spanish advocates brought *Vinifera* grapes to California. Grapes were consumed by Egyptians approximately 6000 years ago, and some ancient Greek philosophers complimented their healing power. Later, Europeans used a lotion made of the sap of grapevines to cure eye and skin diseases; unripe grapes were used to tend sore throats, and dried grapes were used for healing thirst and constipation (Badet, 2011).

Study of the bioactive components of grape seed extracts was first initiated at the beginning of the 20th century. Albert Szent-Gyorgyi, a 1937 Nobel Prize winner, discovered flavonoids while working on the segregation of vitamin C (Szent-Györgyi, 1936), terming them "vitamin P". Subsequently, Professor Jacques Masquelier postulated that because pine bark exhibited ascorbate-like effects, it must contain vitamin C along with flavonoids, which he designated as "pycnogenols", a term no longer used by the scientific community except as a trademark for proanthocyanidins extracted from French maritime pine bark. Masquelier improved and patented a technique to extract oligomeric grape seeds proanthocyanidins in 1947 (Fine, 2000; Murray, 1999), and observed that the bioflavonoids derived from grape seeds appeared to be superior in both concentration and antioxidant effect to those from pine bark (Masquelier, 1991). In 1952, Geissmann and Hinreiner used the term "flavonoids" for the first time, with Bate-Smith and Swain (1962) defining tannins in the following decade.

The proanthocyanidins in wine have been suggested to contribute to the phenomenon termed "French Paradox", whereby a high consumption of dietary fats among the French does not lead to high instances of coronary heart disease and atherosclerosis (Richard, Cambien, & Ducimetiere, 1981). Harbone (1989) categorized the major plant-producing polyphenolic group into 13 subcategories, one of which was proanthocyanidins (Harborne, 2013). The popularity of these compounds has subsequently continually increased in an effort by the general population to recapitulate the French Paradox, accompanied by claims of additional advantageous health effects.

3. Inherent properties of proanthocyanidins

3.1. Structure, chemistry, and biosynthesis

The phenolic combinations in grape seeds consist mostly of flavonoids. Flavan-3-ols make up a large group of flavonoid compounds and are involved in reactions against microbial pathogens, insects, and larger herbivores (Dixon, Xie, & Sharma, 2005). Biosynthesis of polyphenols begins with formation of L-phenylalanine via the shikimate/arogenate pathway. The biosyntheses of flavonols, flavan-3-ols, and anthocyanins share the same upstream pathway (Kobayashi, Ishimaru, Hiraoka, & Honda, 2002). Flavonoids are primarily synthesized in the endoplasmic reticulum and then transported to vacuoles of plant cells, where they finally accumulate in the epidermal cells (Koes, Verweij, & Quattrocchio, 2005). Flavan-3-ols are synthesized prior to flowering (Bogs et al., 2005) and increase until véraison (the transition from berry growth to berry ripening) (Verries et al., 2008). In seeds, the final amount of proanthocyanidins is reached somewhat later than in the skin, a few weeks after véraison (Bogs et al., 2005; Tesniere et al., 2006). The produced polyphenols comprise monomers, oligomers, and polymers or proanthocyanidins (Bogs et al., 2005). All flavonoids are based on a characteristic C6-C3-C6 diphenylpropane skeleton. Major flavan-3-ols monomers in grape comprise (+)-catechin, (−)-epicatechin, and (−)-epicatechin 3-gallate, (−)-epigallocatechin, and trace amounts of (+)-gallocatechin (Fig. 1). Condensed tannins have a complex chemical structure of flavanols. They may contain (epi)catechin, (epi)afzelechin, and (epi)gallocatechin units and they are named procyanidins, propelargonidins, and prodelphinidins respectively. Single linkages i.e., C4-C6 or C4–C8 bonds give rise to B type proanthocyanidins whereas A-type proanthocyanidins show additional C2-O-C7 or C2-O-C5 bonds. Grape proanthocyanidins are essentially of B-type, with C4-C8 linkages being much more abundant than C4-C6 bonds (Monagas, Quintanilla-López, Gómez-Cordovés, Bartolomé, & Lebrón-Aguilar, 2010).

In grape skin and stems; the monomers are (+)-gallocatechin, (−)-epicatechin, (+)-catechin, and (−)-epicatechin 3-O-gallate, (Gu, 2012) whereas seeds are formed by (−)-epicatechin 3-O-gallate, (−)-catechin, and (+)-epicatechin (Souquet, Labarbe, Le Guernevé, Cheynier, & Moutounet, 2000). The content of (+)-catechin and (−)-epicatechin is higher in the colored cultivars than in white grapes (Gođevac et al., 2010). The content of proanthocyanidins at harvest ranges from 0.5 to approximately 6.4 mg/g fresh berry weight in different cultivars (Terrier, Ollé, Verriès, & Cheynier, 2009), reflecting the difference in seed numbers per grape (Harbertson, Kennedy, & Adams, 2002). Differences in proanthocyanidin structures influence the perceived astringency, color stability, and bitterness. The grape variety, geographical and climatic conditions, fertilization, soil, cultivation practices, and degrees of ripeness all influence the proanthocyanidin content (Gođevac et al., 2010). Grape seeds have the highest concentration of bioactive molecules. Approximately 30% of total proanthocyanidins are stored in grape seeds and 15% in skin (Hanlin, Kelm, Wilkinson, & Downey, 2011), although the cell walls need to be broken to allow the proanthocyanidins to be extracted from the skin and seeds (Pinelo, Arnous, & Meyer, 2006). Through the formation of complexes with salivary proteins, proanthocyanidins have a high affinity for proteins, also are responsible for the astringent character of grapes, wine, and cider (Prior & Gu, 2005). However, there is only limited knowledge of the chemistry of proanthocyanidins as analytical methods have generally focused on each oligomer as a class but have been unable to identify the proanthocyanidins within each class (Lin, Sun, Chen, Monagas, & Harnly, 2014). Nevertheless, the occurrence of galloylated oligomeric proanthocyanidins is a characteristic feature of the more highly condensed grape seed polyphenols (Geny, Saucier, Bracco, Daviaud, & Glories, 2003).

3.2. Proanthocyanidin bioavailability, absorption, metabolism, and excretion

The proportion of a compound that is ingested, absorbed, digested, and reaches the systemic circulation is termed its bioavailability (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014). In sequence, the digestive transformations, tissue distribution and bioactivity, intestinal and hepatic metabolism, and absorption into intestinal epithelium cells is referred to as bioaccessibility (Courraud, Charnay, Cristol, Berger, & Avallone, 2013). Thus, the bioavailability rigidly relies on the bioaccessibility activities (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). The bioavailability of proanthocyanidins is highly reliant on the extent of polymerization. The scale of polymerization of a proanthocyanidin molecule is determined by the number of monomeric flavan-3-ol units contained within it. Generally, oligomeric proanthocyanidins are those with a lower degree of polymerization (2–4 monomers), whereas molecules with more than five monomers are termed polymeric proanthocyanidins (Zhao, Pang, & Dixon, 2010). Procyanidin trimers and dimers have been shown to be very steady under duodenal and gastric digestion conditions, with the consumption of dimers being considered to be 100 fold less than that of the monomers (Kumar & Pandey, 2013). It has been demonstrated that skin proanthocyanidins have a higher grade of polymerization than proanthocyanidins extracted from seeds (Eriz, Sanhueza, Roeckel, & Fernández, 2011). Recent studies suggest, however, that only degrees of polymerization lower than 5 are absorbed (Ou & Gu, 2014) or, in the intestinal lumen, further degraded to their flavan-3-ol monomers. Using a distinct enzyme that helps to amplify the extraction of

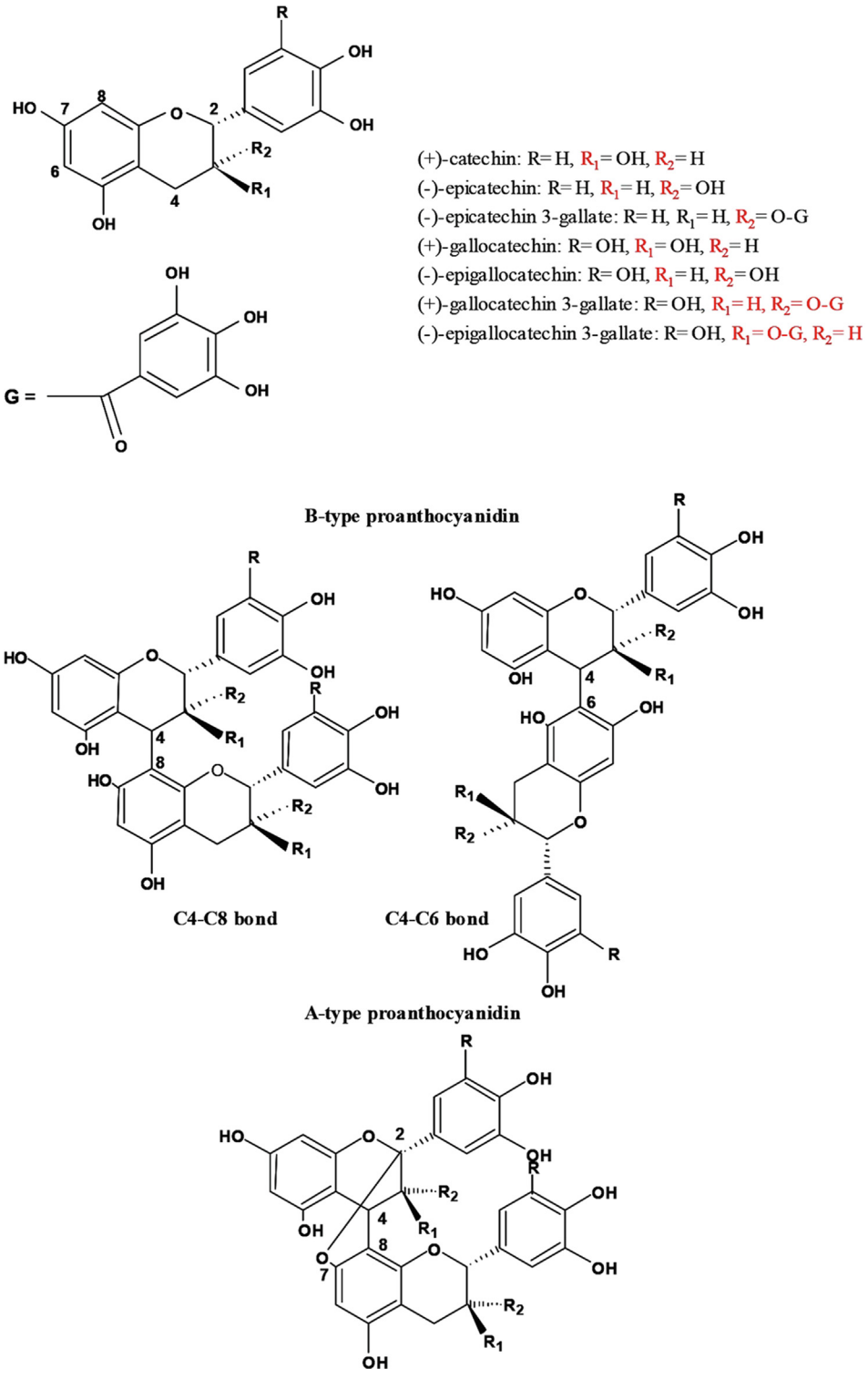

Fig. 1. Structures of grape seed proanthocyanidins.

proanthocyanidins from skin and seed matrices, while at the same time decreasing its typical molecular weight, may constitute an effective method to achieve this contraction (Gu et al., 2004). In addition, protein may have a negative impact on the absorption of proanthocyanidins, although further research is essential to confirm this phenomenon (Ou & Gu, 2014). To date, the polymerization stage of proanthocyanidins remains unsolved.

Proanthocyanidins as antinutrients inhibit digestive enzymes. Some *in vitro* studies indicate that smaller proanthocyanidin polymers and oligomers are consumed in limited amounts using models of the intestinal epithelium, although this uptake is largely constrained to cleaved monomer units (Pappas & Schaich, 2009). Generally, proanthocyanidins are absorbed through passive diffusion (Déprez, Mila, & Scalbert, 1999). Through enterohepatic recirculation, these affixed combinations might subsequently transfer back to the small intestines by means of bile excretion (Romanov-Michailidis et al., 2012).

The biological activities of proanthocyanidins as unaffiliated complicated textures are utilized to regional impact in the gastrointestinal

tract, where they consume metabolites derived from colonic metabolism. Although proanthocyanidins can be dispersed into tissues including the lung, connective tissue, kidney and spleen, most reach the colon in an intact state, preserving intestinal barrier integrity by different mechanisms of action involving their anti-inflammation activity and antioxidant capacity (Choy, Jaggers, Oteiza, & Waterhouse, 2012). Notably, proanthocyanidins can mitigate basic enteral nutrition-induced decreases in luminal small IgAs, which constitute the primary barrier against pathogens (Oteiza, Fraga, Mills, & Taft, 2018). However, whereas proanthocyanidins exhibit important bioactive features *in vitro*, their digestive and metabolic functions *in vivo* are not yet entirely understood (Kruger, Davies, Myburgh, & Lecour, 2014). Finally, proanthocyanidins and their metabolites might be emitted in feces, urine, and bile, or via exhalation following intake of proanthocyanidin-rich diets (Roopchand et al., 2015).

4. Modulated properties of proanthocyanidins

4.1. Analogues and metabolites

The low bioavailability of proanthocyanidins significantly limits the associated health effects because the bioavailability primarily relies on the degree of polymerization. Some new methods have been developed to increase the bioavailability of proanthocyanidins whose dimer equivalents in relation to the C-ring-opened diaryl-propan-2-gallate structural unit display increased antioxidant activities owing to the insertion of one supplementary phenolic hydroxyl. Both proanthocyanidin and its analogues represent a new class of anti-hepatitis B virus agents that target the preS1 area of the anti-hepatitis B large surface protein and act as an inhibitor of hepatitis B disease (Tsukuda et al., 2017).

However, the application of this line of research to the pharmaceutical and food industries is limited owing to the essential formation of phenolic hydroxyl groups, which are easily influenced by oxygen, light exposure, and temperature during product processing and storage. Moreover, proanthocyanidins might be metabolized to dimer and monomer forms, thereby exhibiting high bioavailability in the course of their transport through the intestinal tract and stomach (Zhou et al., 2017). Therefore, the improvement of novel technology to develop durability and resistance to damage would be advantageous. A cage-like protein termed ferritin offers a convenient way to enhance the durability of food bioactive molecules by means of encapsulation nanotechnology. For example, *in vitro* digestion showed that apo-red bean ferritin could extend proanthocyanidin release in an artificial gastrointestinal tract. Additionally, proanthocyanidin compound antioxidant activity was retained to some extent in comparison with that of free proanthocyanidins (Zhou et al., 2017). Proanthocyanidin liposomal suspension could also improve moisture maintenance performance. Proanthocyanidin from the liposome was first released in bursts then by slow release, thus increasing proanthocyanidin shelf life. Finally, a drug delivery system dependent on solid lipid nanoparticles to encapsulate proanthocyanidins provided long-term persistence and stability in the cells (Castellani et al., 2018).

4.2. Gut microbiota

A high proportion of ingested proanthociyanidins are metabolized by gut microbiota prior to digestion. The beneficial health effects are increased with the aid of the proanthocyanidin metabolites formed by the microbiota in the colon. Proanthocyanidins reach the colon via the small intestine, which is the initial site for glucuronidation, and only small amounts are absorbed (Kahle et al., 2007). Both A- and B-types can be metabolized by the gut microbiota. Metabolites include 2-phenylacetic acid, benzoic acid, 3-(3′-hydroxyphenyl) propionic acid, 2-(3′-hydroxypenyl) acetic acid, 3-phenylpropionic acid, 2-(4′-hydroxyphenyl) acetic acid, and hydroxyphenylvaleric acid. Additionally, 5-(3-hydroxyphenyl)-γ-valerolactone and 5-(3,4-dihydroxyphenyl)-γ-valerolactone metabolites have been found in humans (Tzounis et al., 2008). *In vivo* studies indicate that the predominant procyanidin metabolites in urine and blood comprise microbial-extracted phenolic acids and phenylvalerolactone (Ottaviani, Kwik-Uribe, Keen, & Schroeter, 2012).

The gut-extracted microbial metabolites of proanthocyanidins are the main transmitting forms in the blood (Espín, González-Sarrías, & Tomás-Barberán, 2017). The gut microbiota affects food component metabolism and bioavailability and influences metabolic health (Casanova-Martí et al., 2018; Vendrame et al., 2011). Proanthocyanidins promote *Akkermansia muciniphila*, with the bloom rate being dependent on initial intestinal bacterial abundance (Zhang et al., 2018). The numbers of advantageous bacteria such as *Bifidobacterium* and *Lactobacillus* spp. are also significantly increased, although *Clostridium* spp. are constrained (Vendrame et al., 2011).

Owing to several limitations, the potential proanthocyanidin colonic metabolites and complete catabolic pathway are incompletely characterized. The variations in microbiota composition in humans and the limited types of recognized human gut bacteria capable of catabolizing proanthocyanidins and their interactions with proanthocyanidins particularly require further investigation (Bladé et al., 2016; Nash et al., 2018).

4.3. Processing and food matrix effects

An important consideration for the absorption of monomers and proanthocyanidins is the possibility of matrix effects (Vermerris, 2008). Proanthocyanidin levels are undetectable in raisins but high in grapes, indicating that proanthocyanidins are decreased in the course of the drying process (Prior & Gu, 2005). The most impactful method for deriving flavan 3-ols is hot pressing following maceration for 60 min at 60.8 °C, whereas cold pressing without the maceration process is least effective. The combination of catechins decreases in hot pressed juices but increases in cold-pressed juices (Fuleki & Ricardo-da-Silva, 2003). The proanthocyanidin content in grapes degrades 11–16% following heating at 140.8 °C and 100 °C. Freezing and very low temperature procedures effectively inhibit polymeric and oligomeric proanthocyanidin degradation in food substances (Larrauri, Rupérez, & Saura-Calixto, 1997). Because proanthocyanidins mostly exist in the seed coats, seed dehulling should be avoided in mechanical processing of tannin-containing foods. High-pressure treatment could increase proanthocyanidin and decrease epicatechin concentration in grape juice; moreover, heating at 80 °C for 30 min markedly increased proanthocyanidins (He et al., 2016). Thus, processing is essential to increase nutritional value and prolong shelf life (Ahmed & Eun, 2017).

Proanthocyanidins effectively decrease acrylamide in the food matrix. Soaking potato chips in 0.01–1 mg/mL proanthocyanidin from grape seed solutions at room temperature for 15 min prevented acrylamide formation and increased food shelf life and lipid stability while enhancing health-beneficial properties (Sáyago-Ayerdi, Brenes, & Goñi, 2009). Grape seed extract could also be effective as an antioxidant for pre-cooked and frozen beef sausage, based on its sensory characteristics (Kulkarni, DeSantos, Kattamuri, Rossi, & Brewer, 2011), constituting a safe, natural antioxidant for the meat industry.

Fermentation of grapes markedly impacts the amount of extractable proanthocyanidins from the skin and seeds into red wine, whereas white wine is only created from the juice of grapes (Yilmaz & Toledo, 2004). Proanthocyanidins can enhance the capability of wine yeast to defy harmful impact from copper-stress fermentation, and reduce cell metabolic activity (Jia, Liu, Zhan, Li, & Huang, 2015) and fermentation time (Li, Du, Yang, & Huang, 2011).

Owing to their chemical nature to readily bind to fiber, sugar, and protein molecules, most proanthocyanidins remain insoluble (Huemmer & Schereier, 2008). Non-derivable proanthocyanidin may constitute a large part of total proanthocyanidin in food (Serrano et al.,

2009). Two databases are useful for analyzing the proanthocyanidin content in foods for e.g., dietetics, public health nutrition, food technology, and biomedical research: Phenol-Explorer (Neveu et al., 2010) and the USDA database (USDA, 2015).

5. Biological activities of proanthocyanidins

Increasing evidence supports proanthocyanidins as exhibiting beneficial effects against diseases owing to their redox properties, ability to bind target proteins, and modulate cell signaling pathways, which can be defined as a complex cascade of actions that govern the expression changes of specific genes. These pathways regulate various cell processes including growth, proliferation, and apoptosis, with incorrect regulation being linked to cancers, inflammation, and autoimmune diseases. Proanthocyanidins reduce free radical concentration, block their propagation, and chelate metals with their o-diphenol groups (EFSA et al., 2017; Rojas & Brewer, 2007), thus providing significantly greater protection against oxidative stress damage than vitamins C, E, and β-carotene (Han, Shen, & Lou, 2007; Niedzwiecki, Roomi, Kalinovsky, & Rath, 2016). Anti-inflammatory activity action mechanisms consist of modulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways and preventing eicosanoid generating enzymes, inflammatory mediator excretion, and the mitogen-activated protein kinase (MAPK) pathway (Martinez-Micaelo et al., 2012). Grape seeds exhibit important antioxidant capacity to decrease peroxyl radicals, shown by oxygen radical absorbance capacity assay (Huang, Ou, & Prior, 2005) and act on both absorptive and enterohormone-secreting cells in gastrointestinal tract (Pinent et al., 2016). They also show a strong capability to donate electrons using trolox-equivalent antioxidant capacity and ferric reducing ability in plasma assays (Huang et al., 2005). Proanthocyanidins stimulate mitochondrial oxygen use, the electron transport chain, and enzyme action of the citric acid cycle to affect mitochondrial activity for energy augmentation (Pajuelo et al., 2011). This occurs via increased succinate dehydrogenase and Na + -K + -ATPase, and decreased tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) activity in skeletal muscle (Xianchu, Ming, Xiangbin, & Lan, 2018).

5.1. Antimicrobial activity

As rich sources of proanthocyanidins, grape seeds are regarded as novel microbial agents. The high number of hydroxyl groups in proanthocyanidins inhibits bacterial adhesion and coaggregation, reducing biofilm formation and decreasing inflammation. Tannins possess metal ions complexation properties (Cos et al., 2004), especially iron and zinc, which are essential mineral micronutrients for most microorganisms (Daglia, 2012). A-type proanthocyanidins contain an interflavan linkage, which is steadier than B-type linkages (Gu et al., 2003), and thus correlate most robustly with reduced bacterial virulence (Sánchez-Patán et al., 2015).

Defatted grape seed extracts could completely inhibit Gram-positive (*Bacillus cereus, Bacillus coagulans, Bacillus subtilis*) and Gram-negative (*Pseudomonas aeruginosa, Escherichia coli*) bacteria at 850–1000 and 1250–1500 ppm respectively (Jayaprakasha, Singh, & Sakariah, 2001). Grape seed extract further demonstrated antimicrobial activity against *E. coli, P. aeruginosa, Micrococcus luteus, S. aureus, Aspergillus niger,* and *Fusarium oxysporum* with an inhibition growth zone diameter ranging from 15 to 20 mm (Ghouila et al., 2017). Grape seed extracts could act against *S. aureus* after 48 h (Baydar, Sagdic, Ozkan, & Cetin, 2006). The lowest concentration of grape seed extract against *Listeria monocytogenes* was 0.26 mg GAE (gallic acid equivalent)/L, suggesting its potential as a natural antilisterial mixture (Anastasiadi, Chorianopoulos, Nychas, & Haroutounian, 2008).

5.2. Cancer

Cancer resulted in 9.6 million deaths (1 in 6 overall) in 2018 as the second leading cause of death worldwide. Thus, cancer prevention represents a significant public health challenge in the 21st century (WHO, 2018). Fruits and vegetables are assumed as the main dietary factors supporting cancer prevention (Olaku, Ojukwu, Zia, & White, 2015). Most *in vivo* and *in vitro* studies have utilized grape seed proanthocyanidin extracts that exhibit antiproliferative and antiangiogenic effects, and induce apoptosis, cell cycle arrest, and inhibit metastatic processes in the lung (Akhtar, Meeran, Katiyar, & Katiyar, 2009; Xue, Lu, Massie, Qualls, & Mao, 2018), breast (Agarwal, Sharma, Zhao, & Agarwal, 2000; Luan, Liu, Zhong, Yao, & Yu, 2015), colorectum (Derry, Raina, Agarwal, & Agarwal, 2014; Hsu et al., 2009; Kaur, Singh, Gu, Agarwal, & Agarwal, 2006; Nomoto, Iigo, Hamada, Kojima, & Tsuda, 2004; Ravindranathan et al., 2018), prostate (Dhanalakshmi, Agarwal, & Agarwal, 2003; Tyagi, Agarwal, & Agarwal, 2003; Uchino et al., 2010; Vayalil, Mittal, & Katiyar, 2004), liver (Hamza et al., 2018; Joshi, Kuszynski, Bagchi, & Bagchi, 2000), pancreas (Chung et al., 2012; Prasad & Katiyar, 2013), and skin (Vaid, Prasad, Singh, Jones, & Katiyar, 2012).

Grape seed proanthocyanidins can prevent the formation of H_2O_2, protein oxidation, lipid peroxidation, and DNA damage in cells, along with scavenging superoxide anions and hydroxyl radicals, and enhancing the antioxidant defense compounds glutathione peroxidase, superoxide dismutase, catalase, and glutathione (Mantena, Baliga, & Katiyar, 2006). Signal transduction pathway modulation is also crucial in inhibiting the composition of free radicals in a dose-reliant manner (Chen, Liu, & Zheng, 2014; Yang, Tian, Wu, Guo, & Lu, 2018). Proanthocyanidins can bind directly with signaling molecules involved in many cellular processes and regulate their activity. Proanthocyanidins produce both cytotoxic and proapoptotic effects by regulating MAPK and NF-κB-targeted gene expression and antimetastatic impacts by inhibiting genes for cell migration (Uchino et al., 2010). Additionally, lipid nanocarriers containing 25% grape seed oil and 2% laurel leaf oil reached 98% antioxidant activity, evidencing their potential to reduce delivery system toxicity and significantly improve various cellular events and mechanisms; i.e., antioxidant activity, cell cycle arrest and apoptosis induction, and antioxidant enzyme modulation (Lacatusu et al., 2015). These results underline the promise of proanthocyanidins as candidate drugs for cancer treatment and prevention (Cádiz-Gurrea et al., 2017; Nandakumar, Singh, & Katiyar, 2008).

Proanthocyanidin cancer preventive effects also depend on the colonic microbiota (Thilakarathna, Langille, & Rupasinghe, 2018). Dietary supplementation of pigs with proanthocyanidins (1%, w/w) substantially promoted *Ruminococcaceae, Lactobacillus, Lachnospiraceae*, and *Clostridiales* spp. Growth (Choy et al., 2014). Both *Ruminococcaceae* and *Lachnospiraceae* spp. can induce apoptosis by limiting histone deacetylase activity and increasing cellular ROS production via microRNA (miR) 22 expression in hepatic cells (Pant et al., 2017).

5.3. Cardiovascular diseases

Proanthocyanidins exert their cardiovascular protection effects by diminishing lipid peroxidation, blood pressure, plasma homocysteine concentrations, and serum C-reactive protein, and improving hypertriglyceridemia (Pons et al., 2014). Grape seed proanthocyanidins develop lipid homeostasis by enhancing the opposite transport and removal of cholesterol in bile. High oral grape seed proanthocyanidin dose decreased plasma triglycerides and apolipoprotein B and reduced the atherosclerotic risk indication in healthy rats (Del Bas et al., 2005), whereas chronic application reduced dyslipidemia in high-fat diet-fed rats. Grape seed proanthocyanidins cause hypotriglyceridemia by inhibiting lipoprotein secretion rather than enhancing lipoprotein catabolism (Quesada et al., 2012). They also produce some hypolipidemic effects by preventing dietary lipid consumption and reducing

chylomicron excretion by enterocytes (Moreno et al., 2003). Furthermore, repressing very low density lipoprotein excretion in the liver by limiting triglycerides bioavailability also significantly facilitates plasma lipid decrease (Del Bas et al., 2005).

Proanthocyanidin antihypertensive characteristics are associated with delayed endothelial ageing (Oak et al., 2018). Other effects potentially associated with their vasodilator effect include blocking phosphodiesterases 2 and 4, which catalyze cAMP and cGMP degradation, and phosphodiesterase type 5 inhibitor, which degrades cGMP, as well as reducing oxidative stress (Dell'Agli, Galli, Vrhovsek, Mattivi, & Bosisio, 2005). Moreover, AMPK/SIRT1-dependent increased eNOS expression and NO production through KLF2 induction is observed (Cui, Liu, Feng, Zhao, & Gao, 2012). Intake of 300 mg of grape seed extract for six weeks by subjects with mild hypertension reduced systolic and blood pressure by approximately 5.6% and 4.7%, respectively, with a much greater effect observed with the highest initial blood pressure level (Park, Edirisinghe, Choy, Waterhouse, & Burton-Freeman, 2016). However, although proanthocyanidins did not lower blood pressure in middle-aged subjects with pre- and stage I hypertension (Ras et al., 2013), subjects with metabolic syndrome showed positive effect (Sivaprakasapillai, Edirisinghe, Randolph, Steinberg, & Kappagoda, 2009).

By reducing matrix metalloproteinase 2, proanthocyanidins inhibit oxidized low density lipoprotein from linking to lectin-like oxidized LDL receptor-1 and prevent extracellular matrix degeneration. The reduced peroxisome proliferator-activated receptor-gamma causes a reduction in the extent of monocyte–macrophage distinction, with a higher degree of catechin polymerization resulting in stronger inhibition, supporting the cardioprotective role of proanthocyanidins (Mohana, Navin, Jamuna, Sadullah, & Devaraj, 2015).

5.4. Obesity and type 2 diabetes

Proanthocyanidin-rich foods can inhibit the neuropeptides associated with food consumption and satiety by stimulating glucagon-like peptide 1 (GLP-1)/dipeptidylpeptidase 4 (DPP4) activity (G onzález-Abuín et al., 2015). They also inhibit digestive enzymes and suppress fat and glucose consumption from the gut consequent to lipase and amylase inhibition. Factors that effectively influence prevention include galloylation and high type A-linkage percentages, polymerization degree, and monomer proportions during proanthocyanidin formation (Salvadó et al., 2015). Proanthocyanidins in the gut also facilitate gastrointestinal tract-brain signal regulation and incretin-like function (Salvadó et al., 2015). However, their anti-obesity effects may be more attributable to increased energy consumption. Rat diet supplementation with proanthocyanidins resulted in dose-dependent increased adipocyte hyperplasia (Pascual-Serrano, Bladé, Suárez, & Arola-Arnal, 2018), and reduced adipocyte hypertrophy through improved white adipose tissue expansion and body weight gain (Caimari et al., 2015; Pascual-Serrano et al., 2018). Supplementation (0.5 g/kg body weight) did not modify liver gene expression related to lipid oxidation carnitine palmitoyltransferase 1A (CPT1) and non-significantly decreased fatty acid synthesis, whereas 1 g/kg dosing inhibited CPT1, suggesting less ability to oxidize fatty acids in the liver and therefore lower levels of plasma ketone bodies. However, the alternate location of lipid oxidation at the higher dose remains to be determined (Joan Serrano et al., 2017).

Proanthocyanidins also decreased hyperinsulinemia by enhancing adiponectin secretion by white adipocytes and promoting glucose transporter 4 expression in skeletal muscle (Salvadó et al., 2015). They also may inhibit insulin and β-cell mass secretion and formulation (González-Abuín et al., 2015), although discrepancies exist regarding proanthocyanidin effects on body weight, including both reductions and no effect. Therefore, proanthocyanidin antioxidant activity likely decreases obesity-mediated chronic inflammation in various ways: by inhibiting endoplasmic reticulum stress indicators, preventing proinflammatory cytokines, repressing inflammation, inducing metabolic-gene expression by enhancing histone deacetylase action, and mobilizing transcription aspects that alienate chronic inflammation (Chuang & McIntosh, 2011).

Accordingly, proanthocyanidins can facilitate the treatment of diabetes and diabetic complications. Obese rats augmented with various proanthocyanidin concentrations presented dose-reliant hepatic steatosis reduction and decreased miR-122 and miR-33a expression in the liver (Corrêa & Rogero, 2018). Moreover, proanthocyanidins enhance insulin secretion by the β-cell mass and pancreas (González-Abuín et al., 2015). Finally, the gut has also been implicated in proanthocyanidin antihyperglycemic effects by modulating GLP-1 activity levels (González-Abuín et al., 2015). Overall, grape seed proanthocyanidins prevent diabetes by regulating α-glucosidase and lipase activity, reducing anti-inflammatory activity and postprandial glycemia, and improving insulin sensitivity and pancreatic function, associated with increased *Clostridium XIVa*, *Roseburia*, and *Prevotella* (Liu et al., 2017; Zhang et al., 2015).

5.5. Inflammatory bowel disease (IBD)

Subjects with IBD have an increased risk of developing colorectal cancer (Robles et al., 2016). In a rat model, proanthocyanidins extracted from grape seeds caused increased macroscopic damage, mucosal thickness, and villus length, enhanced goblet cell density in relation to increased expression of villin and two key transcription factors, krüppel-like factor-4 (K1f4) and hairy/enhancer of split 1 (Hes1), and enhanced cyclin-dependent kinase inhibitor (P21) content (Li et al., 2011). Consistent with its anti-inflammatory effects, grape seed extract supplementation in the jejunum using IL-10-deficient mice, which are used to model human Crohn's disease, down-regulated NF-κB signaling and reduced TNF-α and IFN-γ expression (Bibi, Kang, Yang, & Zhu, 2016; Wang et al., 2011). Furthermore, total alkaline phosphatase activity decreased, with an associated increase in bowel alkaline phosphatase protein (Bibi et al., 2016). Proanthocyanidin supplementation reduced colonic harm by minimizing pro-inflammatory mediators such as MPO, along with iNOS activity (Wang et al., 2013). Combined with medication, proanthocyanidins markedly reduced IκB kinase activation, causing repression of the phosphorylation-induced degradation of nuclear translocation and IκBα. This may occur by blocking transcription factors signal transducer and activator of transcription STAT3 and STAT1, which are related to cytokine and growth factor receptor formulation (Wang et al., 2011). Bacteroides abundance was also reduced in patients with IBD (Frank et al., 2007). Together with decreased *F. prausnitzii* and increased *Bacteroides* and *Lactobacilli*, grape seed proanthocyanidins thus represent an alternative approach for preventive or therapeutic IBD treatment.

5.6. Neurodegenerative disorders

Proanthocyanidins provide neuronal protection against degenerative diseases by scavenging reactive oxygen species (ROS) (Sutcliffe, Winter, Punessen, & Linseman, 2017). Proanthocyanidins attenuate neurotoxicity and alleviate neurodegeneration in Parkinson's disease cell models by controlling oxidative stress progression and preserving mitochondrial function (Strathearn et al., 2014). The iron-chelating activity of grape seed proanthocyanidin extract minimizes its prooxidant activity and delays 6-hydroxydopamine, a neurotoxin that induces Parkinson's disease (Wu et al., 2010). Notably, extracts rich in proanthocyanidins had greater neuroprotective action than those rich in other polyphenols (Strathearn et al., 2014). Proanthocyanidins significantly increased spatial memory capability, development of amyloid precursor protein and tau protein pathology, and reduced presenilin-1 mRNA expression levels, thus countering oxidative stress in mouse studies (Wang et al., 2012.) A physiologically suitable concentration of 3′-O-Me-EC-Gluc, a biosynthetic brain-targeted proanthocyanidin metabolite, could effectively enhance basal synaptic transmission and

sustenance of lipid transfer protein via mechanisms related to cAMP response element-binding protein signaling activation, a requirement for memory and learning, in Alzheimer' disease. Accordingly, proanthocyanidins provided both increased antioxidant capacity and downregulation of caspase 3-mediated amyloid-beta aggregation and lactic acid dehydrogenase leakage ratio, preventing apoptosis and enhancing the mitochondrial membrane potential (Lian et al., 2016; Wang et al., 2012). Overall, these findings indicate grape seed proanthocyanidin extract as a novel therapeutic agent for treating neurodegenerative disorders.

5.7. Asthma

Administration of grape seed extract decreases the total inflammatory cell and eosinophil numbers (Coleman & Shaw, 2017; Zhou et al., 2011; Zhou, Fang, Zou, Zhang, & Gu, 2015). Combined with medication, proanthocyanidin greatly increases IFN-γ and reduces IL-4 and IL-13 levels, total IgE and T helper cell type 2 (Th2) cytokine levels in serum, and vascular endothelial growth factor levels in lavage fluid (Zhou et al., 2015). Proanthocyanidin also debilitates mucus-producing goblet cells and reduces allergen-caused lung eosinophilic inflammation in the airway. The augmented iNOS expression noted in ovalbumin mice is largely prevented by proanthocyanidin, which decreases the progression of airway inflammation and hyperresponsiveness by downregulating iNOS expression, thus showing potential for treating allergic asthma (Li et al., 2017). Overall, grape seed proanthocyanidins could inhibit airway inflammation and thereby provide a potential treatment for asthma.

5.8. Eye diseases

Proanthocyanidins might shield the eye tissues from oxidative stress, possibly through their antioxidative action by enhancing antioxidant enzymes and reducing prooxidant numbers (Said, Soliman, Azab, & El-Tahawy, 2005). The mechanism of resistance against light-caused retinal degradation likely occurs via molecular metabolite(s) and/or is arbitrated by a far upstream step. Proanthocyanidins could potentially protect human lens epithelial B-3 cells from the harmful effects of oxidative stress. They shield HLE cells from H_2O_2-mediated oxidative stress by decreasing ROS generation and inhibiting NF-κB and MAPK pathway activation (Jia, Song, Zhao, Wang, & Liu, 2011), iNOS, and calpain II in the lenses (Zhang & Hu, 2012). Their potential pharmacological function in reducing H_2O_2-induced oxidative stress implies a defensive effect of grape seed against cataractogenesis (Durukan et al., 2006). These findings suggest that proanthocyanidins might constitute an effective natural agent for inhibiting eye deformity caused by high glucose by restoring *Pax6* (protein coding) expression (Tan et al., 2015). Consequently, future studies should explore which particular structures of proanthocyanidin metabolites are effective in the retina.

5.9. Anti-aging

Proanthocyanidins protect against age-related mental deterioration and depression by inducing hypothalamic-pituitaryadrenal axis action, serotonergic conveyance, and hippocampal neurogenesis (Ogle, Speisman, & Ormerod, 2013). Specifically, proanthocyanidins from grape seed extracts prevented the augmentation of age-related oxidative DNA damage such as 8-hydroxy-20-deoxyguanosine and DNA protein cross-links in diverse brain areas and the spinal cord, and reversed age-related decline in vitamin C levels in aged rats (Jiao, Wei, Chen, Chen, & Zhang, 2017). The antiaging effects are mediated by decreased hepatic and brain thiobarbituric acid responses, along with brain monoamine oxidase actions (Jiao et al., 2017). Proanthocyanidins also elevate Sirtuin 1 expression, which is recognized as an anti-aging agent that extends life span (Yokozawa et al., 2011). Moreover, they possess higher tyrosinase inhibition activity (Hsu et al., 2012) and reduce hyperpigmentation symptoms in female volunteers after a year-long treatment (Jun Yamakoshi et al., 2004). However, further studies regarding specific function and mechanisms are required.

5.10. Osteoarthritis

Proanthocyanidin exerts chondroprotective effects in human chondrocytes (Miller, Bobrowski, Shukla, Gupta, & Haqqi, 2007). Grape seed proanthocyanidin decreases perichondrial inflammation and alveolar bone loss by decreasing matrix metalloproteinase 13 (MMP13), MMP-8, hypoxia-inducible factor 1-alpha (HIF-1α), TNF-α, and IL-17 levels and increasing osteoblastic activity (Toker, Balci Yuce, Lektemur Alpan, Gevrek, & Elmastas, 2018). Grape seed proanthocyanidin extract also reduces the T cell subset levels and upregulates Tregs and Th2 cytokine-producing cell numbers (Ahmad et al., 2013), thus potentially opening up novel avenues for osteoarthritis treatment (Woo et al., 2011).

6. Safe dosage and toxicology

Because of the beneficial effects of grape seed proanthocyanidin to human health, it was examined for toxicological estimation by the National Institute of Environmental Health Sciences, has been certified as generally recognized as safe by the Food and Drug Administration, and is sold as a dietary additive listed on the Everything Added to Food in the United States database. The intense oral toxicity of grape seed extract with 89.3% proanthocyanidins has been determined. The No Observed Adverse Effect Level of grape seed extracts in rats was 1410 mg/(kg weight/day) in males and 1501 mg/(kg weight/day) in females; the LD_{50} of the grape seed extract was > 4 g/kg and no animals died (Yamakoshi, Saito, Kataoka, & Kikuchi, 2002). Oral intake of grape seed extracts up to 400 mg for 12 weeks and 2500 mg for 4 weeks (Sano et al., 2007) was safe and well endured in humans. Proanthocyanidins also served as a safe and effective therapy for pregnant women with condyloma acuminate (Yang, Zhu, Dang, & Zhao, 2016). High duplicated dosing of grape seed powder up to the NOAEL of 4 g/kg or 5% for two months in healthy rats showed that high and continuous dosing of grape seed powder produces anti-inflammatory and antioxidative effects (Charradi et al., 2018). However, more studies need to be conducted in children to examine the tolerance to and safety of proanthocyanidin consumption at the 95th percentile of the aforementioned intake (EFSA et al., 2017). The probable side effects of long-term elevated proanthocyanidin intake; i.e., preventing nutrient consumption, interacting with other food compounds, inhibiting digestive enzymes, and interacting with drugs should be investigated. Thus, more systematic toxicological studies should be administered, given that in the "real world" proanthocyanidins are used as a supplementary element in various food preparations.

7. Conclusions and future directions

Despite the limited number of studies and the difficulties with human intervention trials available, there is a consensus that grape seed proanthocyanidins can contribute to a microbial ecology and modulate gut microbiota and with human health benefits, and thus show promise to use as a nutraceutical. Additional researches are required to fully understand the complex relationship between gut microbiota and grape seed proanthocyanidins to substantiate any potential health benefit claims. Current clinical and epidemiological data show a very preliminary correlation between grape seed proanthocyanidin consumption and health benefits. In some cases, proanthocyanidin intake can reach high levels not usually encountered in the typical diet. Further researches are needed to better investigate the effect of grape seed proanthocyanidins in humans. Given the peculiar pharmacokinetics of grape seed proanthocyanidins, food matrix constituents might exert effects in the gut. Finally, individual genetic variations that could affect

gut uptake and individual microbiota variations can affect the metabolism and, thus, the health effect. All of these aspects should be taken into account about the health effects in humans.

Ethics statement

As it is a review article ethical approval not needed.

Declaration of Competing Interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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