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Grape Seed Nutraceuticals for Disease Prevention: Current Status and Future Prospects

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Abstract

Grapes (*Vitis* spp.) are consumed as fresh table fruits, raisins, and processed into wine, juice, jelly and other value-added products. Grapes contain bioactive secondary metabolites (polyphenols), such as proanthocyanins (oligomeric flavonoids), flavonoids (catechin, epicatechin, and quercetin), and anthocyanins. They have non-flavonoids such as hydroxycinnamic acids (p-coumaric, cinnamic, caffeic, gentisic, ferulic, and vanillic acids), and hydroxybenzoic acids: trihydroxy stilbenes (resveratrol and polydatin). These phytochemicals are of economic importance to pharmaceutical, food and cosmetic industries. Nutraceuticals from grape seeds have potential cardioprotective, anti-cancer, antioxidant, anti-inflammatory, antiviral, neuroprotective, hepatoprotective and antimicrobial properties. Grape seed nutraceuticals have been re-invented in the past few years as a new paradigm in human medicine. In particular, nutraceuticals from grape seeds have been used in stopping wound bleeding, anti-inflammatory agents, pain relief, and anti-diarrhea. In addition, they can be used for the treatment of various human health conditions such as cancer, cholera, smallpox, and nausea as well as eye infections, skin, kidney, liver diseases, etc. Nowadays, consumers are demanding for healthy supplements and personal care products with natural ingredients. Therefore, the present review highlights recent developments and future opportunities of grape seed nutraceuticals for the prevention of human diseases.

Keywords: grape seeds, polyphenols, oxidation, nutraceuticals, human diseases

1. Introduction

Grape (*Vitis* spp.) is one of the most economically important fruit crops worldwide [1]. Grapevine has a rich diversity, as reflected by global variations between wines from different continents or adjacent vineyards. These differences can be attributed to geographical locations, diversity in

climate conditions, or by human interventions arising from breeding and other vineyard management practices. Grapes are consumed fresh as table fruits or raisins and can be processed into wine, jam, jelly, grape seed oil, vinegar, grape seed extracts (GSE) and other products [1, 2]. The quality of grape products depends on a wide range of factors such as variety, environmental conditions, viticultural practices and, more importantly, chemical properties of their secondary metabolites [1]. Secondary metabolites found in grape seeds include phytochemicals such as flavonoids, which are a group of natural polyphenols derived from phenylpropanoid pathway [2]. The stability of secondary metabolites may be impacted by external factors, such as environmental conditions (pH, light, temperature, etc.), due to the nature of physiological functions in growth and development of grapevines [3, 4]. Grape seeds contain phytochemicals such as alkaloids, terpenes, volatile oils, resins, glycosides, tannins, sterols, saponins, and phenolics. These polyphenols have important applications in pharmaceutical, agrochemical, food, and cosmetic industries [1, 5, 6]. More importantly, phenolic compounds are key determinants of wine quality such as aroma, color, and taste and are collectively referred to as organoleptic characteristics of grape products [7, 8]. For example, the quality of grape products is characterized by polyphenolic compounds such as flavonoids and anthocyanins. These natural molecules are generated through specific biosynthetic pathways in grapes.

Flavonoids are primarily located in the epidermal layer of berry skin and seeds in grapes [9]. They have biological attributes such as cytotoxicity and antioxidants, and their biochemical properties play important functions in the defense against biotic and abiotic stresses in grapevines [10, 11]. These phenolic-rich compounds are potential nutraceuticals with biological properties for treatment and/or prevention of various human diseases, whereby they can be used as antioxidants, anti-inflammatory, and antimicrobial agents. Application of nutraceuticals from grape seeds in human health is not new, because wine has been used for medicinal purposes since the medieval period. However, it has reemerged as an important field of human medicine and human nutrition in the past few years [12]. For instance, ethanol in “alcoholic” beverages has potential application in human health due to its properties in inhibition of platelet aggregation, eicosanoid biosynthesis, and an antioxidant for free radicals [13]. Hence, it is important to analyze and characterize grape seed products for biochemical composition, biological activity, and bioavailability of phytochemicals and investigate their correlation to human health.

2. History and establishment of grapes in North America for food and wine industries

Domesticated crops appeared after the Neolithic period (4000–1000 BC) and included grape varieties that were selected for wine production, because they were easier to propagate and had higher proportion of flesh compared to seeds. Wine production from grape has been part of human culture for nearly 6000 years, with the earliest evidence dating back to between 7000 and 5000 BC. Wine produced from grapes was used in dietary applications, medicine, and

socioreligious activities. The earliest known winemaking facility, or at least its development, was considered to be in the Caucasus Mountains (4100–4000 BC), near the village of Areni in Southern Armenia. The region is considered as the origin of domesticated wine grapes (*Vitis vinifera*) [13]. However, the transfer of winemaking knowledge and technology has been somewhat linear, as it moved from Western Asia to Eastern Mediterranean region such as Egypt, Greece, and Southern Italy, before reaching the rest of Europe c.a. 2000 years ago. Today, for instance, wine grape is produced in every continent worldwide, and its chemical composition is profoundly influenced by factors such as improved enological techniques, region-specific grape cultivars, and local climatic conditions [13, 14]. The development of grape production and winemaking methods has been parallel to the overall technological advances, which came after Western civilization. There has been tremendous progress made in grape production, especially with respect to vine cultivation, vinification equipment, and winemaking practices such as fermentation, as compared with those used during the Neolithic era, including contemporary Egypt, ancient Greece, Western Asia, Ancient Israel, or the Roman Empire. These techniques plateaued around 200–400 AD and was followed by a period of between 1200 and 1400 years when the progress of wine technology slowed down and was generally restricted to monastic religious orders of Western Europe [15]. Wine production methods increased during the eighteenth century, and the phenomenon was likely due to positive changes in trade relations in Europe, which led to increased demand for vintage and age-worthy wines associated with higher quality [15].

Wine production began to expand during the seventeenth century, whereby North Americans became latecomers to join the viticulture industry after the arrival of the first European settlers. This was catapulted in part by the quest of the British government to produce its own wine in North America instead of France. The Franciscan missionaries planted the first large-scale vineyards in California c.a. 200 years ago and reestablished them shortly after the repeal of prohibition [16]. However, the “Great French Wine Blight” that originated from California vineyards in the 1850s almost wiped out the European grape production. Grape phylloxera, *Phylloxera vitifoliae*, was transported to Europe on infected California rootstocks, and the pandemic almost wiped out c.a. 2.5 million acres of vineyards in France alone. The tide turned around when vineyards in Europe were replanted with *V. vinifera* grafted on Phylloxera-resistant rootstocks, *V. labrusca*, a fox grape species native to the eastern North America [13].

With the rapid advancement of science, it was only natural that research on grapes with focus on value addition became such a huge subject worldwide. This led to increased demand for improved quality attributes such as wine color, flavor, and chemical composition of phenolic compounds as well as the growth of wine and grape associated-food industries. High demand for vintage and/or age-worthy wines led to increased research in wine and other grape products. The high demand for quality was driven by the fact that wine became an integral part of human lifestyle from various cultural backgrounds worldwide. For example, wine- and grape-related products were used during social events in many countries, whereas in a few other places, these products were used for either religious practices or medicinal purposes.

3. Grape seed polyphenols and their benefits to human health

Grape seeds contain polyphenol-rich phytochemicals such as proanthocyanidins (oligomeric flavonoids), flavonoids (catechin, epicatechin, and quercetin), and anthocyanins. They also contain non-flavonoids such as hydroxycinnamic acids (p-coumaric, cinnamic, caffeic, genistic, ferulic, and vanillic acids) and hydroxybenzoic acids: trihydroxy stilbenes (resveratrol and polydatin) as well as vitamin E [12, 13, 17–19]. These secondary metabolites are responsible for blue, purple, and red colors in many plant tissues. Also, grape seeds are rich in proanthocyanidins, which are oligomeric anthocyanins [20, 21], and a few monomeric flavonoids. Previous studies indicated that these extracts could be used as supplements [22]. The polyphenols from grape seeds are potent antioxidants (**Figure 1**) that can protect the body from wide range of health conditions such as premature aging, numerous diseases, and decay [23]. During winemaking process, polyphenols from grape seeds can also infiltrate into wine products. Therefore, moderate wine consumption has been associated with reduced mortality from coronary heart disease, because it increases high-density lipoprotein (HDL) cholesterol content and, as a result, inhibits platelet aggregation [13]. The antioxidant effects of red wine and its major polyphenols have been demonstrated in many experimental systems spanning from *in vitro* studies (human low-density lipoprotein, liposomes, macrophages, cultured cells) to *in vivo* involving animal models as well as healthy human subjects [13].

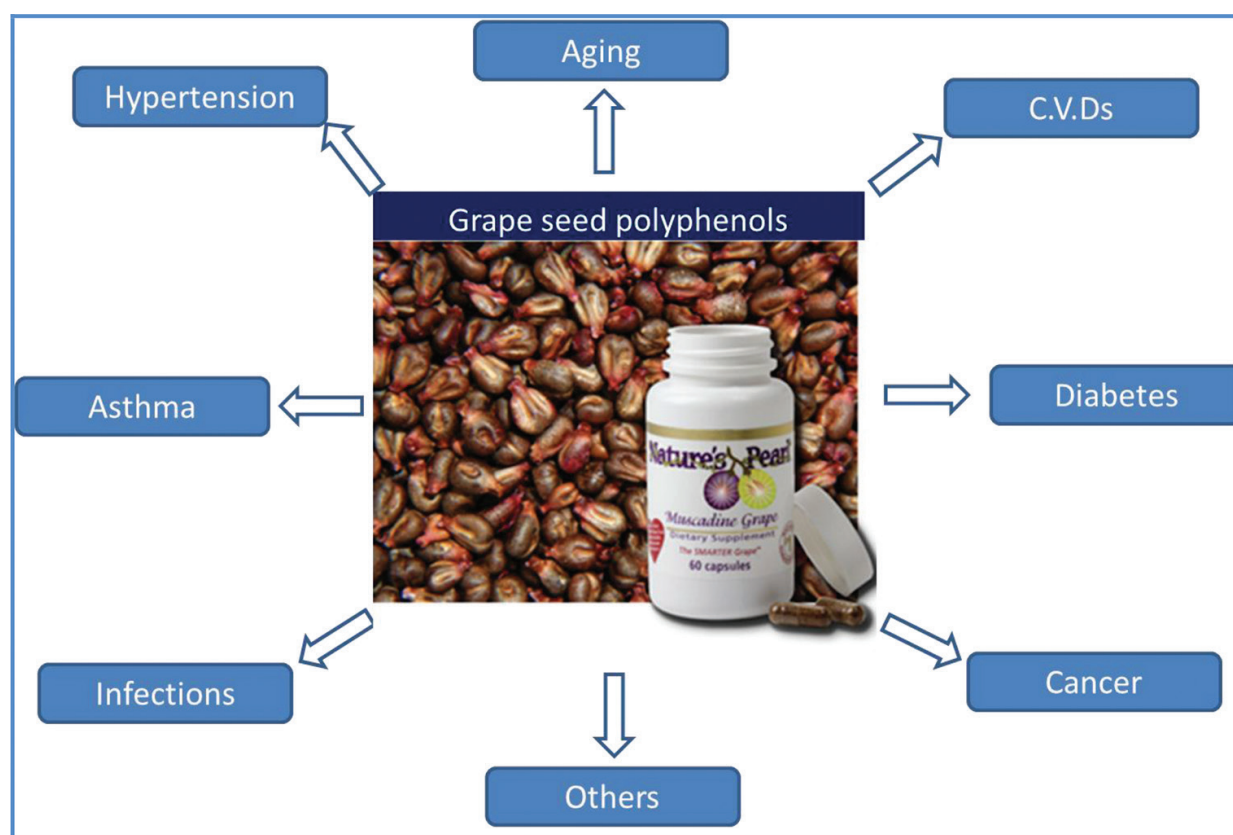


Figure 1. Muscadine grape seeds and their beneficial effects to human health.

4. Chemical composition

Research in the chemical composition of grape and wine has advanced greatly in the past 30–40 years. Currently, more than 500 compounds have been identified from wine, out of which 160 of these phytochemicals are esters. Each individual compound may be either insignificant or has no role in human organoleptic (taste) perception, but collectively contribute to wine taste [13]. In grape seeds, the primary compounds such as water, ethanol, organic acids, sugars, and glycerol are present at high concentrations (>100 mg/L), and the rest are polyphenols. These polyphenols are particularly large and complex group of compounds, which are key determinants of quality of red wines. The identification of phenolic compounds in grapes and wine began in the late nineteenth century [15]. Previous studies indicated that grapes contain phytochemicals with antioxidant properties [16, 23].

Chemically, polyphenols are cyclic benzene compounds that contain one or more hydroxyl groups associated directly with the hydroxy-substituted benzene ring structure such as catechins, resveratrol, cyanidin (**Figure 2**), and proanthocyanidins, which are oligomers of resveratrol, catechin, and epicatechin.

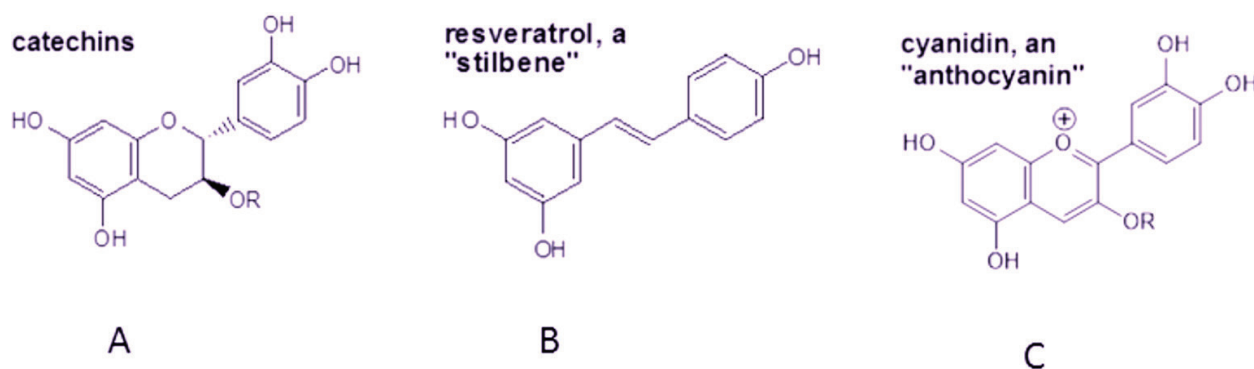


Figure 2. Structures of common healthy compounds found in grape seeds: (A) catechins, (B) resveratrol, and (C) cyanidin.

The two primary groups of polyphenols that occur in grape seeds and wine are flavonoids and non-flavonoids. Flavonoids have been characterized as molecules possessing two phenols, which are joined by a pyran (oxygen containing) carbon ring structure. They have a distinct $C_6-C_3-C_3$ three-ring structure. The most common flavonoids in wine are flavanols (flavan-3-ols), which are the building block of grape tannins; whereas anthocyanins are predominantly present in red wines. Flavan-3-ol monomers (catechin) are responsible for the bitterness in wine and are associated with astringency in wine. The major flavan-3-ol monomers found in grapes and wine include (+)-catechin, (–)-epicatechin, and (–)-epicatechin-3-O-gallate [24]. Flavonoids are primarily from the skins, seeds, and stems of the fruit. Grape tannins, which are polymers of flavanols, are also known as condensed tannins or proanthocyanidins. Proanthocyanidins contribute to the complexity of wine taste and mouthfeel. Flavanol monomers and oligomers (links of two to four monomers) contribute to the bitterness, and their polymers contribute to astringency in wine. There are five anthocyanidins (cyanidin, peonidin, delphinidin, petunidin, and malvidin) in grapes. Anthocyanin with sugar bound

to the anthocyanidin moiety may be acylated (acid linked to the sixth position of the sugar) such as acetic, coumaric, and caffeic acids. Flavonols (kaempferol, quercetin, and myricetin) are present in grape seeds and wine and are esterified to sugars to form glycosides. Flavonols are important cofactors for color enhancement. They also act as a natural sunscreen in the skin of grape berries.

Non-flavonoids have either C_1-C_6 or C_3-C_6 structures, meaning that either one carbon or three carbons are attached to the primary benzene ring (six carbons). The majority of the non-flavonoids found in grapes are phenolic acids: hydroxycinnamic acids or hydroxycinnamates (esterified with tartaric acid: caftaric acid, coutaric acid, and fertaric acid), hydroxybenzoic acids (gallic acid, a hydrolysis product from grape or oak tannins), and stilbenes (resveratrol and piceid). They are predominantly present in pulp of grapes and are the major phenolic compounds in white wine [15]. Previous studies indicated that non-flavonoids are produced in the grape berry before veraison [25]. Polymerization of polyhydroxy flavan-3-ol units, (+)-catechin and (–)-epicatechin, and their gallate esters produces oligomers and polymers called proanthocyanidins. Resveratrol is mainly found in the grape skin, whereas proanthocyanidins are found in the seeds [26]. Previous studies indicated that these compounds are high in seed material [27] and are produced before veraison and change during fruit ripening [15]. Tannins on grapes protect wine against oxidation, stabilize wine color, and enhance mouthfeel [13]. Proanthocyanidins are members of a class of compounds described as anthocyanogens, leucoanthocyanidins, flavan-3,4-diols, condensed tannins, and tannins [15]. Proanthocyanidins are polymers of flavan-3-ol subunits, meaning that they have a wide range of molecular weight. These phenolic compounds mainly impact the astringency in red wines, and they have been extracted from the skin, seeds, and stems of the grape berry. Recent studies demonstrated that proanthocyanidins (flavonoids) are some of the major compounds present in grapes and wines.

Flavonoids are a class of secondary plant phenolics with significant antioxidant and chelating properties. For example, their cardioprotective characteristics stem from their inhibition of lipid peroxidation, chelation of redox-active metals, and attenuation of reactive oxygen species [28]. Primarily flavonoids occur in food polymers that are degraded to variable extents in the digestive tract. Although metabolism of these compounds has remained elusive, it has been established that their enteric absorption is correlated to reduction in reactive oxidative species in blood plasma [28]. The propensity of flavonoids to inhibit free radical activities is mediated by their chemical structure. However, their physical properties such as flavan nucleus, number, positions, and types of substitutions are considered to play a major role in their radical scavenging and chelating activity [28].

Structures of common anthocyanins were identified from *V. vinifera* and were determined wine in 1959. It was noted that malvidin-3-*O*-glucoside was a major grape anthocyanin and was present along with its acylated forms [15]. Similarly, it was noted that anthocyanins from *V. vinifera* showed different structural compositions to those isolated from non-*vinifera* species, because they were exclusively monoglucosides and those in non-*vinifera* species were present as 3,5-diglucosides [15]. Because of the unique hue manifestation of both grape and wine phenolics, several studies have been performed on anthocyanins as compared to other

compounds [15]. These studies have focused on understanding the changes in anthocyanidins in respect to berry development [29], potential impact of cultural practices on production [30], and techniques for their extraction from wine and/or grape seed extracts [31].

5. Biological activities

Grape seed products are nutraceutical agents commonly consumed as a health/dietary supplement or are sold as nutrition supplements (100–500 mg). Grape seeds are rich sources of catechins and procyanidins, which are present in red wine and grape juice. Previous studies indicated that these compounds have shown potent antioxidant activities [32–34] and scavenging activity against free radicals [35–38]. The antioxidant capacity of grape seed extracts is considered to perform better compared with vitamins C and E [39]. It was reported that procyanidins inhibited platelet aggregation [40] and had also successfully inhibited the oxidation of low-density lipoprotein (LDL) as well as contributed to reduction in risks of heart disease associated with atherosclerosis [41]. In addition, procyanidins performed very well as anti-inflammatory [42, 43], antimutagens [44], anticancer [45–47], and antiviral [48] agents.

Grape seed extracts are potential antimicrobial for disease control [49] and can be used in food preservatives to ensure food safety [50]. Currently, there has been growing interest on the use of natural antibacterial compounds, such as herbal-based extracts for the preservation of foods, because they possess a unique characteristic flavor and have shown antioxidant and antimicrobial activities [51]. In general, grape seed extracts can be used as antibiotics, antidiarrhea, antiulcer, and anti-inflammatory agents [52, 53]. For example, the mechanism behind their antiulcer is considered to be due to their ability to protect the stomach wall from injuries caused by free radicals as well as the ability of procyanidins to bind other protein targets [53]. Flavonoids have shown success as antiulcer agents, because their suppressive effect depends on the presence of procyanidin oligomers. Previous studies indicated that procyanidins such as catechin oligomers significantly reduced gastric mucosal damage [53]. Furthermore, the binding ability of procyanidin oligomers to bind bovine serum albumin got strengthened in acidified solution. Thus, understanding the biosynthesis of these phenolic compounds may be important to efficiently manage their production as well as insure their bioavailability after wine production. However, it is important to further explore the *in vivo* potential activities of these secondary metabolites from grape seed extract, to determine their potential pharmacological applications in human medicine.

6. Health benefits

Grape seed extracts have potential to be used as nutraceuticals [46, 54]. For instance, red wine constitutes a reliable and rich source of biologically active phytochemicals, such as phenolic acids and polyphenols, whose individual and summated actions are believed to confer health benefits in humans. Epidemiologic studies revealed that individuals with the habit of daily

moderate wine consumption can experience significant reductions in ill-health conditions, including those leading to cardiovascular mortality as compared with individuals who either abstain from drinking or consume excess alcohol [55]. Moderate consumption of wine, for example, was implicated in reduced atherosclerosis cardiovascular heart disease in the French population [56]. Heart disease is lower among the French (who have a relatively high red wine intake) as compared with other Europeans, despite their propensity to consume foods known to be rich in cholesterol, which was referred to as the “French paradox” due to their low incidence of coronary heart disease. Previous studies determined that drinking one to two glasses of wine a day can help protect against heart disease. In addition, phenolics in grapes and red wines have been demonstrated to inhibit oxidation of human low-density lipoproteins (LDL) *in vitro* [57]. Hence, researchers are working toward understanding both molecular and nutritional basis for these anecdotal observations. Although the benefits of polyphenols from fruits and vegetables is gaining ground on how they are making significant contribution to human health, but consensus toward increasing the rate of wine consumption is developing quite slowly [55]. This could be due to the negative perception that a huge segment of the society has toward alcohol consumption.

Recently, plant polyphenols have generated increased attention due to their potent antioxidant properties and ability to prevent various diseases associated with oxidative stress [58]. This led to identification and development of phenolic compounds or extracts from different plants with health and related medical applications in the past few years. Medicinal and nutritional values of grapes have been known to exist for thousands of years. For example, several ancient Greek philosophers exalted the healing power of wine from grapes, and in places, such as Ancient Egypt, wine was mythologically considered as “The Gift of Osiris.” They also used wine as a solvent for other medicinal products and in combination with other medicines (“polypharmacy”), and wine has been used in prescriptions dating back to between 3400 and 2550 BC [59]. More importantly, wine was at the core of Mediterranean civilization and was the basis of vast seaborne trade that contributed to the spread of Greek civilization beginning the sixth century, including western Asia Minor, southern Italy, Sicily, North Africa, southern France, and Spain [59]. Wine was held in high esteem by the Persians because of its fame as a cure, which was epitomized by the oldest desert proverb: “He that has health has hope; and he that has hope, has everything.” Through the medieval period, the conversion of grapes into wine was considered as a gift from the gods, and the best wines were mainly preserved for ruling elite of the society, although the latter may have been associated with the high cost of wine. In addition, wine was used for medicinal purposes during the medieval period in areas such as ancient India in 2000 BC, ancient China in the past 5000 years, and ancient Rome after 146 BC as well as during the era of Biblical Jewish and Arabic period [59]. The rich history of wine provides an insight of its benefit to human health for several years. It is, therefore, the reason why modern science continues to explore benefits of grape seeds as nutraceuticals with the potential to revolutionize modern human medicine.

To date, various parts of grape are known to confer therapeutic benefits in humans. For example, grape leaves can be used to alleviate wound bleeding, inflammation, muscle pain, and diarrhea; whereas unripe grapefruits are recommended for the treatment of sore throat.

Raisins (dried grape fruits) can be used to provide relief against constipation and are recommended for treatment of human diseases, including anemia in pregnant women. In addition, ripe and sweet grapes can be used for treatment of diseases such as cancer, cholera, smallpox, nausea, and eye infections as well as can provide relief against skin, kidney, and liver disorders [23]. Grape extracts are rich in bioflavonoids (procyanidins) and are some of the most commonly consumed dietary supplements in the United States due to their potential health benefits. Previous studies indicated that grape extracts are beneficial to human health, because they have showed some efficacy against several diseases such as prostate carcinoma, which causes prostate cancer in men; cardiovascular-related conditions such as hypertension; vascular fragility; and other health conditions such as allergies, hypercholesterolemia, LDL-cholesterol oxidation, and platelet aggregation disorder [60–63]. Other studies have showed that grape extracts can be used as therapeutic agents against diabetic cardiomyopathy [64].

Phenolic compounds such as catechin, quercetin, and resveratrol been known to promote the production of nitric oxide by vascular endothelium cells and, as a result, inhibit biosynthesis of thromboxane in platelets and leukotriene in neutrophils demonstrated to modulate biosynthesis and secretion of lipoproteins in animal models and human cell lines, and arrest tumorigenesis and subsequent carcinogenesis [13]. However, lack of statistical correlation between wine consumption and lower rates of atherosclerosis has made it difficult to resolve the key question of whether moderate consumption may likely lead to decreased atherosclerotic mortality [55]. However, some studies have suggested that there is a relationship between wine consumption and atherosclerotic mortality, which may likely be associated with its direct effect on lipoprotein metabolism.

Moderate ethanol intake is not generally contraindicated in diabetes, although diabetic patients have showed high risk for atherosclerotic cardiovascular disease [65], initiated from oxidative damage of glycosylated proteins and lipids [66–68]. Thus, diabetics as well as nondiabetics are likely to benefit from moderate ethanol intake, because it can help ameliorate risk factors associated with atherosclerotic heart disease that arise from lipoprotein metabolism. Although little information is available on the effects of polyphenols in wine and related grape seed products on diabetics, there may be confounding lifestyle variables associated with spontaneous wine consumption, such as reduced smoking, increased exercise frequency, and better dietary habits that mimic general recommendations given to diabetic patients [55].

7. Bioavailability

Some flavonoids found in wine, such as tannins, are in the form of polymers that may not readily break down under physiological conditions and may not be expected to be available for absorption [69]. Conversely, for non-tannin flavonoids in red wine, about half are present as glycosides, and the rest are present as aglycones (glycosyl group replaced by hydrogen). It has been shown that flavan-3-ols, flavonols, anthocyanins, and non-flavonoid stilbenes, which are

present in red wine and related grape products, can be absorbed in the gastrointestinal tract [55]. Previous studies reported that anthocyanin pigments, which are responsible for the red color of wine, were found in human urine [70] and human plasma [71] after wine consumption. Most glycosides that pass into the large intestine generally end up being hydrolyzed by fecal microflora, rendering them as free aglycones, which leads to their poor absorption in the small intestines. Resveratrol, a non-flavonoid trihydroxystilbene, which is a relatively minor component of red wine [72], is present at insignificant low levels in white wines [55, 73]. However, long-term wine consumption can increase tissue-specific resveratrol concentrations in the body [74].

Flavonoids can travel in the body while bound to plasma proteins [75, 76]. The metabolism of flavonoid glycosides to aglycones and specific glucuronides occurs in the intestinal tissue, which depends on the structure of the flavonoid [77]. The metabolism of esterified flavonoids to aglycones leads to increased lack of absorption in the small intestine. However, conjugated derivatives of quercetin have retained partial antioxidant activity, and it has been demonstrated that different combinations of these conjugates have potential additive effects [78]. Phenolic acids and polyphenols possess multiple hydroxyl groups and are subject to further metabolism by enzymes in the intestine, liver, and kidney [79, 80]. However, the main drawback is that other important nutraceuticals may not be fully absorbed in the human body. Besides, bioavailability under *in vivo* conditions needs to be resolved as well as their absorption in the gastrointestinal tract in order to keep them at sustainable pharmacokinetic levels in the blood system.

8. Safety concerns for application of grape seed products from American native grapes in foods and cosmetics

Today, consumer demand for health supplements as well as personal care products with natural and/or organic ingredients is promising. Consumption of a large amount of grapes and related products, such as wine, has contributed to the low risk of chronic diseases, such as coronary heart disease and certain cancers [81]. Grape seed extract, which is primarily a mixture of proanthocyanidins, has been shown to modulate a wide range of biological, pharmacological, and toxicological effects as well as cytoprotective functions. Previous studies investigated the ability of IH636 from grape seed proanthocyanidin extract (GSPE) for the prevention of acetaminophen (AAP)-induced nephrotoxicity, amiodarone (AMI)-induced lung toxicity, and doxorubicin (DOX)-induced cardiotoxicity in mice [82]. However, there are safety concerns on the use of these natural products in humans.

Probably one of the best-known properties of polyphenolic compounds, which may also be of safety concern, is their binding and precipitation of protein targets [52]. Most polyphenols have the capacity to bind proteins because of their high affinity for hydroxylation, that is, introduction of hydroxyl groups on other essential biological compounds. Conversely, low-molecular-weight phenols lack the ability to precipitate proteins, unless their oligomeric composition has at least three flavonol subunits, which may mainly be found on highly

polymerized tannin molecules. Tannin-protein complexes arise from interactions between hydrogen molecules and hydrophobic moieties without the participation of their respective covalent or ionic bonds [52]. The safety of plant products may vary due to several factors such as geographical origin, growth conditions, and other production processes [83]. It is therefore important to conduct studies to characterize these phytochemicals from plant-based products, in order to investigate whether they are safe for use by humans.

To date, there are no conclusive studies on contraindication of grape products for either treatment or prevention of human diseases as well as their use in cosmetics. However, recent studies suggest that grape seed products may be safe for treatment of important human diseases. For example, in one particular study which involved the administration of grape seed extract (GSE) as a dietary admixture at levels of 0.02, 0.2, and 2% (w/w) to rats for 90 days, it was demonstrated that GSE showed no toxicity. Results indicated lack of toxicity, which supported the use of proanthocyanidin-rich grape seed extracts [22]. Similarly, in a different study that the administration of GSE IH636 to male and female Sprague-Dawley rats in the feed at levels of 0.5, 1.0, or 2.0% for 90 days, it was reported there were no significant toxicological effects [84]. Besides, Wren and colleagues found no significant changes in clinical signs, hematological parameters, organ weights, ophthalmology evaluations, or histopathology, and identical results were reported from a different study that was conducted on IGS BR rats, which were fed with dietary supplements of GSE at concentrations of 0, 0.63, 1.25, or 2.5% (w/w), which found lack of adverse effects on mouse models [85].

These studies pointed to lack of toxicity and supported the use of proanthocyanidin-rich extracts from grape seeds, except for personal care products, in which there has been some evidence for minimal side effects on the skin surface such as irritation, sensitization, phototoxicity, and immediate-type allergy [86].

9. Conclusions and future remarks

Phenolic compounds found in grape seeds have desirable biological activities, which are related to their antioxidant properties. These compounds have the ability to scavenge for free radicals and inhibit non-desirable enzymatic activities, including the modulation of essential biosynthetic pathways for the metabolism of cellular and extracellular products such as membrane proteins and lipids. Moderate consumption of red wine, for example, can lead to increased plasma concentrations of HDL cholesterol and decreased adhesion of platelets, which may be beneficial to treating platelet aggregation in humans. Biosynthesis of pro-aggregatory eicosanoids such as thromboxane A_2 and synthesis of leukocytes by pro-inflammatory leukotrienes to inhibit the formation of atherosclerosis can reduce the risk of coronary arterial disease by promoting the relaxation of vascular smooth muscle [16, 87]. In addition, some polyphenols are capable of promoting the synthesis of prostacyclin (prostaglandin member of eicosanoids) and nitric oxide, which can lead to optimized blood flow through the arterial system [13].

Use of nutraceuticals from grape seeds for the mitigation of grape diseases for which biomarkers are known have not been validated. Although nutraceuticals have shown a great promise in the treatment and/or prevention of human diseases, consensus on their wide acceptance as alternative therapeutic agents is developing very slowly. Previous studies demonstrated that nutraceuticals found in grape seeds likely have the ability to modulate cellular metabolism and signaling, which is consistent with reduced coronary arterial disease [16, 87]. However, additional research is needed to address crucial important issues such as the mechanisms by which phytochemicals in grape seeds can be used as nutraceuticals, develop biomarkers for their role in disease prevention, and determine the appropriate dosage for their application in human health medicine.

There are still several gaps that need to be addressed. First, there should be a broad framework for research to understand the mechanism by which polyphenols from grape seed products confer dietary health benefits to humans. Second, additional clinical studies are needed to determine their pharmacokinetic properties in human disease prevention. Third, appropriate biomarkers or mechanistic end points should be developed to determine the interactions between diseases and polyphenols at cellular and subcellular levels. Lack of adequate clinical data on nutraceuticals from grape seed products precludes their use in human health. First, it is not known whether the benefit of red wine originates from the tendency to consume them during meal time, or their consumption together with other macronutrients improves their absorption in the gastrointestinal tract. Second, it is not known whether the ability of wine to scavenge for free radicals originates from its chemical properties as a biofuel, through its ability to cause oxidative stress, or using both mechanisms, which may suggest that it has multiple effects at the cellular or subcellular level.

The success of human genome project and recent developments in molecular biology has led to major revolution in biological sciences. Thus, the application nutraceuticals from grape seeds to human health need to be prioritized as one of the areas of current research. Additional research should focus on understanding potential effects of confounding factors that may be associated with the use of nutraceuticals from grape seeds. This is because preliminary data suggest that nutraceuticals from grape seeds have valuable applications in the prevention and/or treatment of several human diseases. Hence, there are unlimited opportunities presented by the application of polyphenols from grape seeds as nutraceuticals. Therefore, it is important to investigate not only their bioavailability and chemical composition but also to characterize their biological properties to determine their mechanisms as well as their synergistic properties and other components of human diet.

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