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REVIEW

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Anthocyanins from Pomegranate (*Punica granatum* L.) and Their Role in Antioxidant Capacities *in Vitro*

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As phytochemicals, anthocyanins are not only responsible for the diverse colors in nature, but are associated with broad-spectrum health-promoting effects for human beings. Pomegranate is abundant in anthocyanins which possess high antioxidant capacities. However, the pomegranate anthocyanins profile and their contributions to antioxidant capacities are not fully depicted. The purpose of this article is to review anthocyanins from pomegranate as important antioxidants. Total anthocyanin content (TAC) and six major components vary greatly with intrinsic and extrinsic factors. In pomegranate, anthocyanins mainly acted as primary antioxidants, while their action as secondary antioxidants were not conclusive. The antioxidant potentials of anthocyanins were significantly affected by factors especially chemical structure and detection assays *in vitro*. The current knowledge may provide insights into potential applications for pomegranate anthocyanins based on their antioxidant activities.

Keywords: pomegranate, anthocyanins, antioxidant capacities, *in vitro*.

1. Introduction

Pomegranate (*Punica granatum* L.), a member of Lythraceae family,[1] is native to Central Asian areas including Iran, Afghanistan, Pakistan, and is one of the most known ancient fruit trees and traditional medicinal plants in the world. Due to its extensive adaptability to climate and soil conditions, the tree has been widely planted in the Mediterranean region, Asia, California of the US, Chile of Southern America, and Africa. Pomegranate is a versatile species with high economic, ornamental, nutritional and pharmaceutical values but in recent years, more investigators are interested in its medicinal and health-care functions. Owing to the presence of a very broad array of natural polyphenols compound such as anthocyanins, flavonoids, punicalagin, and ellagitannin, pomegranate has been widely used in folk and modern medicine.[2] According to avilable laboratory-based studies, disease targets of pomegranate include cancer, cardiovascular disease, inflammation, diabetes, hyperlipidemia, obesity, and oral disease.[3] Consequently, a growing public awareness of the potential health implications of pomegranate has promoted the high demand for fruits and other related products worldwide.

More than 500 pomegranate cultivars have been named around the world, which are characterized by the high genetic diversity of morphological and biochemical quality traits.[4] Of these, color phenotype is one of the distinguishing features of pomegranate cultivars. The large variations in fruit and flower color among different cultivars in pomegranate are mainly attributable to the anthocyanin-derived pigments. As one of the widely distributed secondary metabolites in higher plants, the anthocyanins are responsible for red coloration in flowers, fruits, and leaves, and play a key role in the special unique physiological functions, such as the prevention of photo-oxidative damage, facilitation of pollination and seed dispersal, protection against various abiotic stress.[5] Furthermore, anthocyanins are known for their antioxidant activities which scavenge free radicals and offer protection against

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age-related degenerative diseases and other chronic disorders.[6,7] Therefore, there is an ever-increasing interest in the consumption of antioxidants based on natural resources like anthocyanins.

Pomegranate contains high levels of anthocyanins, mostly occurring in the peels, arils, flowers, and leaves.[8–13] Nevertheless, scientific studies lack sufficient attention paid to anthocyanins compared to other polyphenols. Despite the fact that Bar-Ya'akov et al.[14] encapsulated the differences in their published review with regard to the composition and concentration of anthocyanins in pomegranate according to both genetic and environmental conditions, they did not cast light on the relationships between anthocyanins and their antioxidant potentials. Therefore, in order to develop a better understanding of the anthocyanins and their contribution to antioxidant capacities of pomegranate, this review collects and collates the reported anthocyanin-related compounds in pomegranate, highlights variations in anthocyanin associated with factors such as plant tissues, cultivars, developmental stages, and environmental conditions, with a special focus on antioxidant properties exerted by pomegranate anthocyanins.

2. Diversity of Color and Anthocyanins in Pomegranate

2.1. Color Diversity in Pomegranate

Pomegranate is diversified especially in fruit and flower colors. The fruit skin color ranges from yellow to purple, with pink and red most common, while aril color ranging from white to dark red. The flower color of table pomegranate is either red or white, while the color of ornamental flowers ranges from white, pink, orange and red to multicolor (*Figure 1*). Also, new leaves of the plant are transiently red because of the accumulation of anthocyanin pigments.

2.2. Chemical Diversity of Anthocyanins in Pomegranate

2.2.1. Structure of Anthocyanins in Plants

Anthocyanins, belonging to flavonoids family, are natural hydro-soluble pigments that are responsible for the intense red to blue colors of various plants. Structurally, all anthocyanins have an identical flavonoid C6-C3-C6 skeleton, which is also referred as 2-phenyl-benzopyrylium cation or flavylium,[5] a system of two aromatic rings (A and B) joined by a C-ring with two double bonds, hence carry a positive charge (*Figure 2*). Chemically, anthocyanins have glycosidic structure bound to the aglycon, the anthocyanidin. The glycosides combining of one or more sugar groups in aglycones could confer stability and water solubility of the molecules, therefore, anthocyanidins rarely occur naturally in the form of aglycones. The most common sugar moieties, which are mainly bound to the molecule at the C3-position of the C-ring or the C5- or C7-position of the A-ring, are glucose; nevertheless galactose, arabinose, rhamnose, and xylose may also occur.[15]

It is considered that more than 8000 different anthocyanins in land plants.[17] To date, chemical studies have documented over 700 anthocyanins in diverse plant species, and more than 30 individual anthocyanidins have been identified in nature,[18] but only six of them are frequently occurring: cyanidin (Cy), delphinidin (Dp), pelargonidin (Pg), petunidin (Pt), peonidin (Pn) and malvidin (Mv) (*Table 1*). The number and position of the hydroxy, glycosyl, acyl and phenylacyl moieties have been reported as the major decoration forms for anthocyanins, leading to the multitude of anthocyanins known today.[19] In search

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Zhaohe Yuan graduated from Nanjing Forestry University and worked in Shandong Academy of Agricultural Sciences. He received his Ph.D. degree in Shandong Agricultural University in 2007. In 2013 he entered into Nanjing Forestry University and engaged in germplasm evaluation and genetic breeding of pomegranate.

Figure 1. The color diversity in pomegranate fruits and flowers.

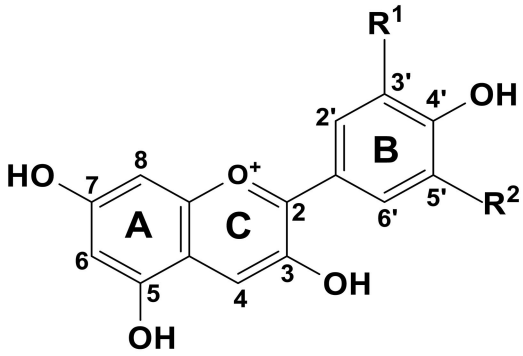

Figure 2. Structure formula of anthocyanidins (according to Kähkönen and Heinonen[16]).

Table 1. Six common anthocyanins in higher plants.

Anthocyanidin	R^1	R^2	Molecular weight	Visible color
Pelargonidin (Pg)	H	H	271	orange
Cyanidin (Cy)	OH	H	287	orange-red
Delphinidin (Dp)	OH	OH	303	purple
Peonidin (Pn)	OCH_3	H	301	orange-red
Petunidin (Pt)	OCH_3	OH	317	purple
Malvidin (Mv)	OCH_3	OCH_3	331	blue-red

of major anthocyanins for each aglycone and decoration moiety in the KNApSAcK database (http://nanaya.naist.jp/KNApSAcK/),[20] which is one of the largest phytochemicals databases, a total of 461 anthocyanins for each aglycone and decoration moiety (Cy, 161; Dp, 94; Pg, 90; Mv, 53; Pn, 33; Pt, 30) are found. In fact, approximately 90% of anthocyanins are based on Cy, Dp, Pg, and their methylated derivatives.[5] Of these compounds, cyanidin 3-glucoside (Cy3G) is the most widely distributed anthocyanin species in edible plants such as pigmented fruits and vegetables.[21]

2.2.2. Diversity of Anthocyanins in Pomegranate

Presently, a large variety of anthocyanins have been identified in different parts of the pomegranate trees, especially in fruit peels and arils, flowers, and leaves. Typically, the anthocyanin components in pomegranate include 3-glucosides and 3,5-diglucosides of Cy, Dp, and Pg,[8,11,22] namely cyanidin 3-glucoside (Cy3G), cyanidin 3,5-diglucoside (Cy3,5dG), delphinidin 3-glucoside (Dp3G), delphinidin 3,5-diglucoside (Dp3,5dG), pelargonidin 3-glucoside (Pg3G), pelargonidin 3,5-

diglucoside Pg3,5dG) (*Figure 3*). The relative proportions of different groups of anthocyanins determine the intensity of color, thus causing colorful phenotypes. More than 100 anthocyanidin and anthocyanin compounds are identified in pomegranate, which are presented in Table S1. On the whole, the variations of anthocyanins are extensive and induce both qualitative and quantitative changes in the pattern across different genotypes,[12,13] tissues,[14,23,24] extraction method,[25–27] abiotic conditions such as developmental stages,[9,11,28,29] climate changes,[11,28,30] sunlight exposure,[31] and saline conditions.[32] These factors, ranging from intrinsic genetic to various extrinsic environmental and their interactions, determine the anthocyanin accumulation among cultivars temporally and spatially.

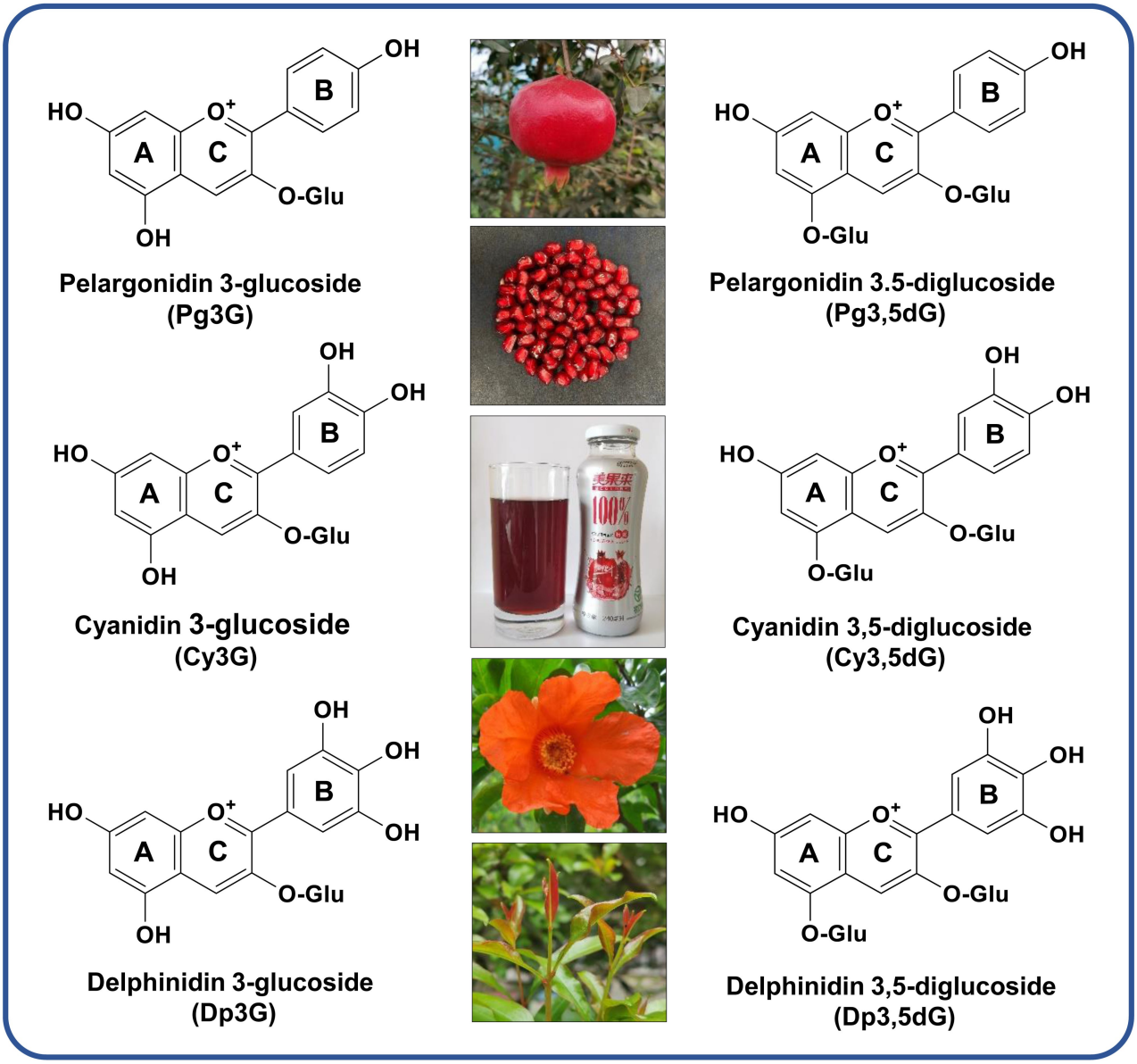

Figure 3. The six commonly distributed anthocyanins in pomegranate.

3. Variation of Anthocyanins in Pomegranate

3.1. Variation of Anthocyanins in Different Pomegranate Parts

3.1.1. Anthocyanins in Pomegranate Fruit Arils/ Juices

The pomegranate is consumed worldwide exclusively as fresh fruit and juice, though jam, jelly, and nutritional supplements may also occur. The edible part of the fruit is called aril and constitutes approximately 50% of the total fruit (w/w).[33] The aril color varies largely among cultivars, ranging from white, pink to red and dark red. The appealing color of aril is one of the most important sensory attributes and, perhaps, nutritional advantages of pomegranate. The tightly packed juicy arils of pomegranate are a rich source of anthocyanins, which concentration has a range of 29.9%–73.2% of the total phenolics in juice varying with cultivars.[22]

It is generally acknowledged that there are six major anthocyanins, Cy3G, Cy3,5dG, Dp3G, Dp3,5dG, Pg3G, and Pg3,5dG in pomegranate juice,[9,12,23,25,34–38] albeit with some differences of opinion. The cultivar is one of the most important factors which influences anthocyanin profiles. For example, Pg3,5dG was not identified in a commercial Not from Concentrate (NFC) juice[39] and aril juice of Croatia accessions.[40] Pg3G was absent in aril juices of Indian cultivars,[41] whereas only four types of anthocyanins Dp3G, Dp3,5dG, Cy3,5dG, and Pg3,5Dg were ascertained in Indian ecotypes[42] and six Spanish cultivars.[43] In 'Wonderful' pomegranate[9] and four Spanish clones,[29] Cy3G was detected as major pigment. The content of six individual anthocyanins presented a great variability in 30 Tunisian cultivars, with Cy3,5dG 3.1–74.4 $mg \cdot L^{-1}$, Dp 3G 0.7–22.0 $mg \cdot L^{-1}$, Cy3G 0.8–21.0 $mg \cdot L^{-1}$, Pg 0.5–16.1 $mg \cdot L^{-1}$, Pg3,5dG 0.0–11.8 $mg \cdot L^{-1}$, and Dp3,5dG 0.0–5.4 $mg \cdot L^{-1}$.[44]

In the juice obtained from the whole fruit, some colored flavanol-anthocyanin[45–48] and anthocyanin-flavanol[49] adducts were identified and quantified. These compounds were present at very low concentrations, with a content lower than 1.7% of total anthocyanin content.[48] The concentration of anthocyanin-flavanol was much lower even when compared with those of flavanol-anthocyanin adducts.[49] These data suggest a more complex profile of anthocyanins in whole-fruit juice.

3.1.2. Anthocyanins in Pomegranate Fruit Skin

Skin color is an important quality parameter attributing the marketing acceptability, because the fruits with brilliant red coloration produced by anthocyanins tend to have a more aesthetic appeal to consumers. Additionally, the anthocyanins accumulate in the outer peels attribute to the nutritional value of commercial pomegranate juice with their entry into the juice during industrial processing.

Numerous studies have confirmed that the fruit peel showed a high concentration of polyphenolic compounds, hydrolysable tannins, flavonoids, and anthocyanins being the representative ones. Anthocyanins are ranked as compounds responsible for bioactivity in pomegranate peel extract, though lower contents are recorded than hydrolysable tannins.[50] Different researchers have come to different conclusions regarding the composition and concentration of anthocyanins in fruit peels. As reported by Russo et al.,[22] anthocyanins were below the limit of detection in peel samples in Italian varieties, though six anthocyanins were detected in juices. The detected anthocyanins among the Tunisian pomegranate peels were identified as Cy and Pg mono-substituted anthocyanidin derivatives, including pelargonidin-3-pentoside, cyanidin-3-glucose, rutinoside, and pentoside derivatives.[51] Four anthocyanins, including mono- and di-glucoside of Cy and Pg were determined in the peels of two Israel cultivars P.G.116-17 and P.G.200-211[52] and 'Mollar' pomegranate.[9] In two Israel accessions P.G.135-36 and P.G.100-1, most of the anthocyanins in developing fruit skin were Cy derivatives, while Pg and Dp pigments were at a comparatively low level.[53] Previous studies on three distinct Chinese cultivars evidenced 6 major anthocyanins variation in fruit peel, with the results that Cy3G was dominating pigment in red and green cultivar, while Cy3G and Dp3G were predominate in dark red cultivar.[11] Of seven cultivars from south Africa, Cy3,5dG and Dp3,5dG were detected in cultivar 'Arakta', 'Bhagwa', and 'Herskawitz', while only Cy3,5dG was detected in 'Ganesh', 'Ruby', and 'Wonderful' cultivars, no anthocyanins were identified in 'Molla' fruit peel.[50] In general, the quality and quantity of anthocyanins in peels are as diverse as that in arils, which are significantly influenced by genetic background.

3.1.3. Anthocyanins in Pomegranate Flowers, Leaves and Seeds

In flowers, only two anthocyanins, Pg3G and Pg3,5dG, were identified in a Chinese cultivar 'Daqingpitian',[10] Contrary to that report, Yisimayili et al.[54] found five anthocyanins in dried flowers, namely Cy3G, Cy3,5dG, Dp3,5dG, Pg3G, and Pg3,5dG. Four anthocyanins, i.e., Cy3,5dG, Dp3G, Pg3G, Pg3,5dG were detected in 'Wonderful' pomegranate flowers,[53,55] while only Cy3,5dG and Pg3,5dG were detected in 'Valenciana' flowers.[55] Quantitatively, these data confirmed that principal pelargonidins (orange color), lower levels of cyanidins (red color) and negligible amounts of delphinidins were accumulated in flowers of pomegranate, with Pg3,5dG being the most concentrated.

Bekir et al., who quantified anthocyanins in pomegranate leaves collected in May, found that with the polarity increase of extracting solvent, the concentration of anthocyanins in leaves decreased. Of the five extraction systems, the hexane extract contained the highest total anthocyanin content (TAC), while the values obtained in the ethanol extract was found to be the poorest.[56] From mature leaves collected in October, Arlotta et al. identified only two (Dp3,5dG and Cy3,5dG) in 'Valenciana' and three (Dp3,5dG, Dp3G and Cy3,5dG) kinds of anthocyanins in 'Wonderful' pomegranate, respectively.[55] These findings demonstrated that the pomegranate leaves may contain specific anthocyanins, albeit at a much lower level. However, there are no reports documenting the anthocyanins from the newly-sprouted colored-leaves.

Owning to the absence of attractive red colors, the seeds are generally not to be a subject study of anthocyanins. It is true that no anthocyanins are detected in seeds.[55] When the only small amount of TAC was found in seed extract,[57,58] it was presumed that the detected anthocyanins were from those remaining from the juiced arils.

3.1.4. Comparative Analysis of Anthocyanins in Edible and Non-Edible Parts

Comparative studies are conducive to recognize and understand the healthy phytochemicals among different pomegranate parts. A generally accepted view is that both peel and juice are rich sources of anthocyanins. Higher levels of tannins are certainly observed in pomegranate peels than that in juices,[22,23,58,59] while for anthocyanins, researchers retain diverse opinions towards it. As reported by Orak et al., the juice extract included approximately 7-fold higher TAC than that in peel extract according to mean values.[58] Of the four pomegranate parts such as flowers, leaves, juice, and peel, juice extract showed the highest level of TAC, followed by flower and peel extracts, and the lowest value of anthocyanins was recorded in leaf extract.[24] The concentration of anthocyanins in pomegranate fractions was also been found to be higher in aril extract than in peel,[23,58,60,61] indicating that the pomegranate peel is not rich in anthocyanins compared with aril juice.[58] However, Elfalleh et al. recognized that TAC was more abundant in peel than in juice.[62] The different anthocyanin fingerprints may result from genetic heterogeneity in aril juice and peel, and other parameters such as ecotypes, harvest maturity, storage conditions, and the position on the tree. Despite the contradictions in the TAC content between aril juices and peels, in most cases, various anthocyanins are characterized in pomegranate juice, while hydrolytic tannins are featured in fruit peel.

3.2. Variation of Anthocyanins in Different Cultivars

The cultivar genotype is the main factor responsible for the variability of pomegranate TAC. Numerous reported data have covered a large range of values for TAC both in peels and in juices.[22,25,34,36,40,43,48,62–76] For most cultivars, the quantitatively dominant anthocyanin components are Cy3G and Cy3,5dG, accounting for more than 85% of total anthocyanins,[13,29,38,77] but there are often different descriptions in different cultivars. As proof, Dp3,5dG and Dp3G were characteristic pigments in 13 Tunisian pomegranate juices.[76] In Italian genotypes, there were reports that Cy3,5dG and Pg3,5dG,[78] Cy3,5dG and Dp3,5dG,[31,55] Cy3G and Pg3,5dG[22] were defined as the most representative anthocyanins, respectively. Higher amounts of Cy3G and Dp3G were found in red commercial genotypes from India.[74] In Iranian cultivars[36,79] and four Spainish cultivars Cy3,5dG and Dp3,5dG were both identified as featured anthocyanins.[43] Therefore, it can be inferred that genetic make-up of pomegranate is the determinant for the concentration and composition of anthocyanins.

3.3. Variation of Anthocyanins in Different Developmental Stages

For red pomegranate, it is generally accepted that the content of anthocyanins increases continuously and attain a maximum value in the mature stage, despite fluctuations in individual anthocyanins. According to

the results of Fawole and Opara,[38] the Cy3G and Cy3,5dG evolved considerably as fruit ripeness progressed, suggesting a more important role of the two anthocyanins played for juice coloration during maturation. Another study showed that in the early developmental stages of Spanish clones, Dy3,5dG was the main pigment in juice, followed by Cy3,5dG, while in the later stages, the 3-glucosides of Cy and Dp increased considerably.[29] Similarly, results were proved that the amount of 3,5-diglucosides was higher than that of 3-glucosides during the first fruit development stages, and that in early maturation stages the amount of Dp glucosides were higher than Cy glycosides, while in the later maturity stages the Cy glycosides were the main pigments of the fruit juice, and the 3-glucosides reached similar or higher concentrations than the 3,5-diglucosides.[9,29] It can be concluded that ripened fruits are known to contain higher mono-glucosides than di-glucosides but much smaller amounts of Pg derivatives.[29,74] All these results reveal that major compositional changes of anthocyanins in pomegranate are developmental regulated.

3.4. Variation of Anthocyanins with Environmental Conditions

Environmental conditions, especially sunlight intensity and temperature, significantly affect pomegranate fruit quality and health beneficial compounds.[80] Knowledge of how environmental conditions affect anthocyanins synthesis has not yet been established, but the overall results provide valuable information for evaluating the impact of environmental changes on fluctuations of anthocyanins.

Di Stefano et al. reported the effects of sun exposure on anthocyanin and non-anthocyanin phenolic levels in pomegranate juices.[31] The results revealed that significantly higher anthocyanin content was found in juices with South and North exposure, while reduced concentration of flavonoids and phenolics in fruits exposed to sun radiation in South, East, and West positions. Hence, the authors suggested that collecting fruits with different solar exposure could obtain different health benefits of fruit juice.[31]

Temperature is the primary environmental factor affecting anthocyanin content and aril color. TAC was up to 45-fold higher in the aril juice of the fruit grown in the Mediterranean region than that in the desert climate, suggesting that the relatively lower temperature favors the production of anthocyanins in aril.[81] This might be a consequence of anthocyanin degradation and color loss at high temperatures.[82] Moreover, it was found that the degree of anthocyanin glucosylation was highly dependent on the temperature conditions, since an increased proportion of diglucosylated anthocyanins were monitored with seasonal warming.[28] The cool temperature during fruit ripeness may enhance the accumulation of delphinidins,[28] which suggested that delphinidins were more temperature-liable than cyanidins. This can be explained by the fact that the intensity of the red color is inversely related to the sum of heat units during fruit development.[83] Therefore, it deserves serious consideration with regard to how to create a favorable environment for fruits production to yield better sensory appeal of color.

3.5. Variation of Anthocyanins with the Extraction Procedure

In different pomegranate parts including juice, peel, flower, and carpellary membrane, several extraction methods have been developed. The juice extraction techniques and their impacts on juice quality have been adequately reviewed by Hegazi et al.[84] As a matter of fact, the extraction procedure, including extraction methods and solvent types, could be a major factor affecting the concentration of anthocyanin compounds. Take fruit juice as an example, the commercial pomegranate juice usually contains higher levels of anthocyanins than juice from arils, due to the entrance of anthocyanins from the peel into the juice.[60,75] Parashar et al. evaluated quality and stability of pomegranate juice using two extraction methods: one was separation of seeds from fruits and centrifugation, the other was squeeze of fruit halves with an electric squeezer.[85] No significant differences were found in the content of anthocyanins in juices obtained through two extraction methods, though the drastic decrease of Cy3,5dG content obtained by the former method.[85] In another study, maceration of arils resulted in a reduction of TAC compared with the other two juice extraction methods.[86] Therefore, the concentration of anthocyanins of the same cultivar varied with extraction methods.

The classical organic solvent extraction method holds an important position in TAC availability. Among other contributing factors, the solvent is crucial to extracting efficiency. The most commonly used solvents for pomegranate anthocyanin extraction are hydroalcoholic solutions containing ethanol and methanol,[87] and ethanol is preferred over methanol due to its safety and easily removal during the subsequent evaporation process.[26] Considerable

quantities of anthocyanins were obtained in the extraction with ethanol and aqueous fraction, whereas no anthocyanins were detected when ethyl acetate was used for both whole fruits and peels.[26] Elfalleh et al. reported that methanol and water were both effective for extraction of anthocyanins, though methanol extract was shown a higher concentration of TAC.[27] By analyzing different ecotypes using different extraction solvents, Abid et al. presented that the highest value of anthocyanins was achieved in 'Acide' ecotype with acetone, 'Gabsi' ecotype with ethanol extract, and both 'Nebli' and 'Tounsi' ecotypes with water extract.[51] It can be noticed that novel methods, such as Superheated Solvent Extraction (SSE) and instant Controlled Pressure Drop (DIC) assisted Solvent Extraction (DIC-SE) methods were also applied for the availability of polyphenols including anthocyanins.[88] Compared with conventional solvent extraction methods, SSE and DIC were more effective for extraction yield, which the TAC was increased by 35.5% and 18.8%, respectively.[88] To conclude, the yield of extraction extremely depends on the solvent polarity. In order to obtain the maximum yield of anthocyanins, extraction solvent types should be seriously considered according to different pomegranate tissue samples and cultivars.

4. The Role of Anthocyanins in the Antioxidative Capacities of Pomegranate

Dietary antioxidants, including anthocyanins, are believed to be effective nutrients in the prevention of oxidative stress-related diseases. An antioxidant is able to scavenge free radicals and suppress lipid peroxidation, so as to decrease or eliminate the cumulative oxidative damage in the body. Antioxidant capacity is an important and fundamental function in living systems. Many significant progresses have recently been made towards assessment of the antioxidant potentials in phytochemicals. As a rich source of anthocyanins, pomegranate is suggested to have strong antioxidant capacities. For instance, when the antioxidant activity was detected before and after removing the anthocyanin fraction from pomegranate juice, Gil et al. found that the remaining phenolic compounds only accounted for 28% of the total activity, indicating the importance of anthocyanins for the antioxidant effects of pomegranate fruits. Yet, positive and negative correlations between pomegranate anthocyanins and antioxidant capacities frequently occur among different study groups.[60] Indeed, antioxidant activities determined in various samples are influenced by several factors. Discrepancies with measured antioxidant activity in pomegranate may arise from cultivars,[22,51,63,68,69,89] extraction procedures,[27,51,56] and test system applied.[69,90] Of the variables, the chemical structure of anthocyanins and the *in vitro* detection protocols are important contributing factors determining anthocyanin antioxidant capacities.

4.1. The Antioxidant Mechanism of Anthocyanins

The autoxidation process is typically chain reactions that involve the initiation, propagation, and termination of free radicals (*Figure 4*). From a mechanistic standpoint, antioxidants can be generally categorized into primary and secondary types.[91,92] Primary antioxidants or chain-breaking antioxidants, are known to be radical scavengers by donating their reactive hydrogen to the peroxy free radicals to form products thereby break radical chain sequences. Secondary antioxidants, also known as hydroperoxide decomposers, react with hydroperoxides to yield non-reactive products (*Figure 4*).[92,93] Two categories are often used in combination to yield synergistic stabilization effects. Antioxidants have been reported to work through single or combined mechanisms, which include free radical scavenging, reducing activity, complexing of pro-oxidant, scavenging lipid peroxyl radicals, and quenching of single oxygen,[91] that is, anthocyanins may act as radical scavengers, reducing agents, hydrogen donators, metal chelators, and also singlet oxygen quenchers. Flavonoids, which are usually regarded as strong antioxidants, can exhibit their antioxidant activity mainly in three ways: radical scavenging activity; metal chelation activity; and interaction with other antioxidants.[94] The majority of previous studies have tried to unravel the antioxidant mechanisms of anthocyanins from 2 perspectives: the scavenging of radicals and the prevention of radical formation by the chelation of metals, especially iron.[58] It was reported that the pomegranate peel had low chelating activity despite its important radical scavenging activity,[51] indicating that pomegranate peel possesses high primary antioxidant activities, but low secondary antioxidant activities. Orak et al., on the other hand, demonstrated that pomegranate peel, juice and seed all exhibited metal chelating capacity, which was significantly affected by factors such as the solvent and cultivar.[58] Therefore, it still remains unclear about the antioxidative capacity of pomegranate evaluated by metal chelation activity.

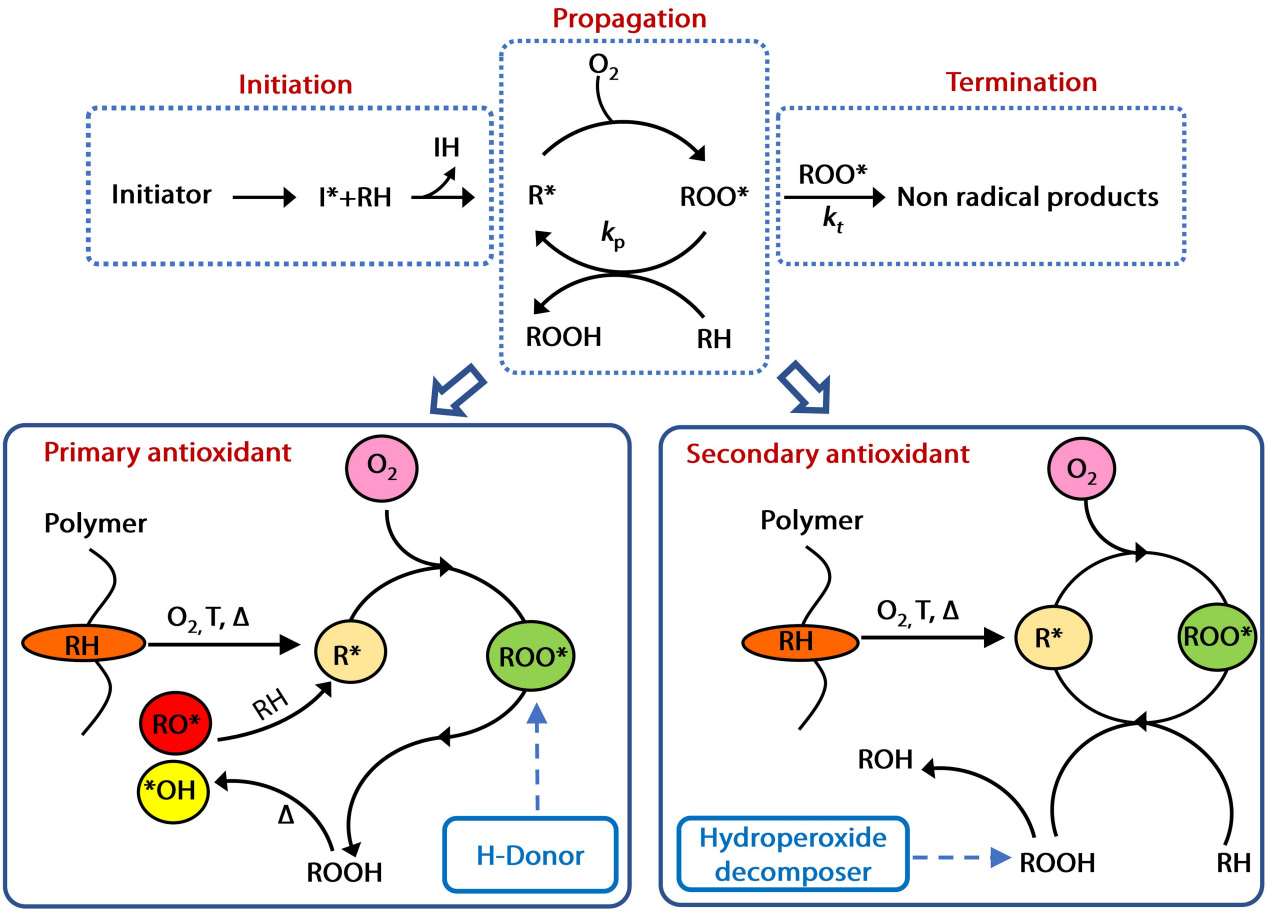

Figure 4. Chain reaction of oxidation and proposed antioxidative mechanism (According to Amorati and Valgimigli[93]).

4.2. Structure-Antioxidant Relationships of Anthocyanins in Pomegranate

The antioxidant properties of anthocyanin are closely associated with their chemical structure. The structure-activity is linked with numerous factors, including positions, numbers and types of chemical groups, such as hydroxy, methyl, acyl, and glycosyl on the anthocyanin aromatic rings. For flavonoids, three structural requirements as illustrated in *Figure 5*, including the catechol (ortho-dihydroxy) group in the B-ring, the double bond conjugated with the 4-oxo group in C-ring, and the hydroxy groups in 3- and 5- position, are important for their high antioxidant activities.[94–96] For anthocyanins, due to the absence of the double bond in C2-C3 and 4-oxo group, the substituents present in the B-ring significantly affect the antioxidant capacities of anthocyanins, whose contribution to the efficiency followed the order of –OH > –OCH3 > –H.[97] The B-ring 3′,4′-dihydroxy configuration is of primary importance in determining the antioxidant activity of anthocyanins for pomegranate (*Figure 5*).[96]

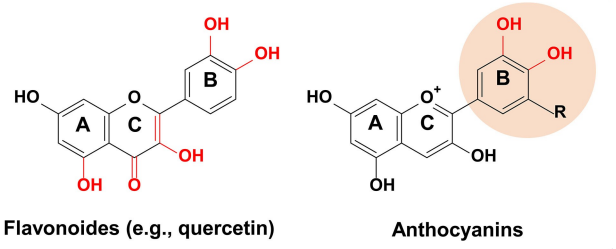

Figure 5. Functional groups playing a key role in antioxidant capacity in flavonoides and anthocyanins.

4.2.1. Hydroxy Groups-Hydroxylation

The configuration, substitution, and the total number of hydroxy groups substantially influence antioxidant activities. The number of hydroxy groups in C3′ and C4′ position of ring B are vital for free radical

scavenging activities of anthocyanins.[98] It was reported a more powerful scavenging radical activity in Dp than in Cy,[16,99,100] which was attributed to the presence of three hydroxy groups on the B-ring of Dp. However, a contrary result was reported by Wang et al., who found that purified Dp with three OH on the B-ring had a low oxygen radical absorbance capacity (ORAC) activity than Cy, Pg, Mv, and Pn compounds with one or two hydroxy groups.[101] The authors suggested that the hydroxy groups on the 3′ and 4′ positions in the B-ring were important determinants for the radical scavenging potential in anthocyanins, while the 5′-hydroxylation appeared to decrease the ORAC activity in the presence of 3′ and 4′-OH. Pg, Mv, and Pn with only one –OH group in the B-ring had lower activities compared to Cy and Dp with more than one –OH substitution assessed by ORAC, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays.[16,101,102] These results demonstrated that the structure 3′- 4′ catechol was highly determinant in scavenger activity.[95]

4.2.2. Sugar Groups-Glycosylation

Different glycosylation patterns either enhance or diminish the antioxidant power of anthocyanins,[16] which depend on the position, degree, and sugar groups of glycosylation and anthocyanidins. The anthocyanins generally decrease antioxidant capacity when compared to their corresponding aglycone.[103] However, this was not always the case. Wang et al. reported that glucosylation either increased (Cy3G *vs.* Cy), decreased (Mv3G *vs.* Mv), or did not have a significant effect (Pg *vs.* Pg3G) on the ORAC activity of the aglycones.[101] Results from the DPPH assay showed that the activities of Cy, Dp, and Mv 3-glucosides were almost the same as their corresponding aglycones, whereas the mono-glucosides of Pn and Pg were less active than their aglycones, Pt3G possessed higher radical scavenging activity than aglycone Pt in the DPPH test.[16]

The quality and quantity of the sugar substituent affected the antioxidant activity as well. Wang et al. reported an increased ORAC activity of Cy for glucose and rhamnoglucose but decreased activity for galactose substituent.[101] Likewise, Cy-galactoside and rutinoside were weaker scavengers than the glucosides by DPPH assay.[16] The anthocyanins having glucose and galactose monosaccharides showed higher antioxidant capacities than those containing disaccharides.[16,17] These results were confirmed by Zhang et al., who demonstrated a higher antioxidant activity exerted by Pg3G than by Pg3,5dG in pomegranate flowers.[10]

4.2.3. Other Structural Modifications

Other structural modifications, such as O-methylation and acylation of hydroxy groups of anthocyanins also play important parts on the observed antioxidant activity. The presence of methylation at C3′, C4′ and C3 positions generally reduce the antioxidant benefits of anthocyanins.[17] The Mv and malvidin 3-gluccoside (Mv3G), which have two –OCHS substituents, were found to have a lower antiradical capacity and reducing capacity than their anthocyanin counterparts Cy and Dp, Cy3G and Dp3G, which have no –CHS groups.[99] This conclusion was not reached in the studies of Kähkönen and Heinonen, who reported in their DPPH assays that Pn, having a methoxy group in the 3′-position, was more active than Pg, having no methoxy group in 3′-position.[16] In addition, a study carried out by Sintzing et al. exhibited a decrease of ORAC value by 5-glycosylation and an increase by acylation of antioxidant potency, respectively.[104]

4.2.4. Structure-Metal Chelation Activity

Metal chelation is widely considered as another mechanism of the antioxidant activity of flavonoids. Metal chelation activity of an antioxidant is referred to as prevention of the transition metal-catalyzed production of reactive species.[94] Structurally, the metal chelating capacity is ascribed to the hydroxy and carbonyl groups in the structure of flavonoids, with the catechol moiety to be the major contributor.[96] Take taxifolin, a kind of flavanonol as example, the proposed metal ion binding sites were the catechol in ring B, the carbonyl in ring C, the 3- and 5-hydroxy groups in ring C and ring A.[96] Nevertheless, it was reported that there was no positive correlation between metal-chelating capacity and TAC in different pomegranate parts including peel, juice, and seed, though a higher metal-chelating capacity was observed in peel extracts than juice and seed extracts.[58] It is supposed that glycosylation of anthocyanidins, especially for Cy, Dp, and Pt components, in the 3-hydroxy or 5-hydroxy position might decrease the metal-chelating capacity. However, more studies are needed to confirm these suggested functional chelation sites and chelating capacity, since it is proposed that the anthocyanins with their 3′,4′-dihydroxy groups can quickly chelate metal ions to form stable antho-

cyanin metal complexes.[17] The negligible metal chelating activity was also observed in methanol extract and punicalagin of pith and carpellary membrane of pomegranate fruit though it contained many phenolic OH groups in the structure.[105] Therefore, the metal chelating activity defined by the structure of anthocyanins in pomegranate merits further elucidation.

It can conclude that antioxidant activity is assigned to the presence of functional groups in anthocyanin structure, especially the catechol moiety. Different hydroxylation and glycosylation may modulate their antioxidative properties, which depend largely on the aglycones of anthocyanins.

4.3. Measurement of Antioxidant Activities of Anthocyanins in Pomegranate

Recent interests in antioxidants due to their involvement in health benefits have led to the development of many *in vitro* assays for detection of antioxidant capacity. Depending upon the reactions involved, radical scavenging action may be broadly classified as electron transfer (ET) and hydrogen atom transfer (HAT) based assays (*Table 2*).[94,96,106,107] HAT methods measure the ability of an antioxidant to quench free radicals by hydrogen donation, commonly used ORAC and total radical trapping antioxidant parameter (TRAP) assay being included in this category. ET methods measure the ability of a potential antioxidant to transfer one electron to reduce radicals, metals, or carboyls, ferric ions reducing antioxidant power (FRAP), total phenols contents (TPC) when using Folin-Ciocalteu reagent are included in the category.[96,106] In fact, ET and HAT reactions almost always occur together in all samples but with different rates, and the mechanism finally dominating in a system is determined by the antioxidant characteristics.[108,109] Hence, mixed-mode assays, such as DPPH, Trolox equivalent antioxidant capacity (TEAC), and ABTS are regarded as the third category of *in vitro* assays.[110] Since the antioxidant mechanisms are so complex in the biological matrix that it is reasonable to apply different antioxidant assays to obtain reliable results.[111] While these techniques present discrepancies and do not reproduce exactly *in vivo* conditions, nevertheless, they allow classification and estimation of antioxidant activity.

Table 2. *In vitro* methods most commonly used to evaluate antioxidant capacity of anthocyanins.

Method	Reaction mechanism
Total radical-trapping antioxidant parameter (TRAP) assay	HAT
Oxygen radical absorbance capacity (ORAC) assay	HAT
Total oxyradical scavenging capacity (TOSC) assay	HAT
Total phenols contents assay by Folin -Ciocalteu reagent	ET
Ferric-reducing antioxidant power (FRAP) assay	ET
total reducing capacity (TRC)	ET
Cupric ion reducing antioxidant capacity (CUPRAC) assay	ET
N,N-dimethyl-p-phenylenediamine dihydrochloride (DMPD) radical scavenging assay	ET
1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay	ET or HAT
2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)/Trolox-equivalent antioxidant capacity (TEAC) assay	ET or HAT

Abbreviations: ET, electron transfer; HAT, hydrogen atom transfer

Published articles cover a series of methods in determining the antioxidant activities of pomegranate anthocyanins, some most frequently used are free radical scavenging assays like DPPH, ABTS, and FRAP assay, which are based on spectroscopic techniques (*Table 3*). With different in vitro experiments used, different results can be obtained. Elfalleh et al. found that TAC showed a strong correlation with FRAP and ORAC values, rather than DPPH and ABTS.[67] Based on the FRAP and ORAC assays, TAC was correlated with the antioxidant activity in juice only detected by ORAC, not by FRAP.[66] In a study of fruit juices and homogenates prepared from 29 pomegranate accessions, it was found an insignificant correlation between TAC and antioxidant activity by DPPH but a significant correlation by FRAP.[75] By using the same 11 cultivars and the same FRAP methods, Borochov-Neori et al.[83] and Schwartz et al.[81] obtained diametrically opposed results. The former believed that the antioxidantive capacity was linearly correlated with TPC, while the latter concluded that the antioxidant levels were positively correlated with TAC. It is speculated that the minor changes in the reaction system, and the subsequent uncertainty surrounding the chemistry of anthocyanin compounds, may lead to the discrepancies. Therefore, comparison of the absolute values with the ones reported in other articles is difficult.

A large percentage of findings in pomegranate demonstrated an excellent correlation between TPC

Table 3. Reported studies on evaluating the antioxidant capacities of anthocyanins in pomegranate.

No.	Samples	Antioxidant assays	Key findings	Reference
1	Flowers from Chinese 'Daqingpi' cultivar	DPPH; ABTS	-The antioxidant activity of Pg3G stronger than that of Pg3,5dG.	[10]
2	Fruits of Israeli and Italian origin	NBT; ABTS; DPPH; ORAC	-A strong correlation between scavenging activity and TPC ($r=0.97$), as well as TFC ($r=0.92$) by ORAC. A less relevance was observed between TAC and the antioxidant activity by the four assays.	[26]
3	15 accessions from Spain	ABTS; DPPH, FRAP	-TAC was highly correlated with all assays (r = 0.72** by ABTS, r = 0.73** by DPPH, r = 0.69** by FRAP) in aril juice. No significant correlations of total punicalagins and vitamin C were found.	[34]
4	'Mollar' from Spain	DPPH; ABTS; FRAP	-TAC was strongly correlated with ABTS (r = 0.8617**), DPPH (0.7180**) and FRAP (0.9187**) assays in juice.	[37]
5	Not from concentrate (NFC) juice	TEAC; ABTS; ESR method scavenging activity against galvinoxyl and DPPH radicals; the potential ROS reduction using HepG2 cells.	-Anthocyanin and copigment fraction exhibited scavenging activity against galvinoxyl and DPPH radicals in a dose-dependent manner. Anthocyanins and copigment fraction showed significant protective effects against H_2O_2-induced toxicity in HepG2 cells.	[39]
6	8 pomegranate accessions collected from Croatia	DPPH; TOSC	-Antioxidant activity was correlated with TPC ($r=0.820$) by DPPH but not with TAC.	[40]
7	One variety 'Hicaznar', three genotypes 19–121,17–67,19–66 from Turkey	DPPH; β-Carotene bleaching assay; reducing power; metal chelating capacity	-DPPH scavenging activity significantly correlated with the level of TAC (r = 0.479**), TPC (r = 0.528**), TTC (r = 0.441**), and acidity (r = 0.689**) in juice, negatively and insignificantly correlated with TAC in peel. β-carotene bleaching activity correlated to TAC (r = 0.381*) and acidity (r = 0.838**) in juice, and peel extracts (r = 0.460**, 0.504**, respectively). Reducing power correlated with TAC (r = 0.503**), TPC (r = 0.512**), TTC (r = 0.391*), and acidity (r = 0.586**) in juice. While in peel, no significant correlation with TAC was registered. Metal chelating capacity was not positively correlated with TAC, TPC, TTC, acidity in juice. In peel, the correlation coefficients were 0.610** (TPC), −0.217*(TAC), 0.528**(TFC), 0.374* (TTC).	[58]
8	Four types of 'Wonderful' juice, 2 were fresh aril juice, 2 were commercial juice	ABTS; DPPH; DMPD; FRAP	-The activity was higher in commercial juices than in experimental ones. The main antioxidant compounds in juice were punicalagin and other hydrolysable tannins, but anthocyanins and ellagic acid derivatives also contribute to the total antioxidant capacity of the juice.	[60]
9	Mature pomegranates from Tunisia	ABTS; DPPH; Reducing power	-In peel, TPC, TFC, and o-diphenols were significant correlated with antioxidant capacities revealed by DPPH and reducing	[62]

Table 3. (cont.)

No.	Samples	Antioxidant assays	Key findings	Reference
			power. No significant correlations were found between TAC and total antioxidant capacities in flower.	
10	8 cultivars from Turkey	ABTS; DPPH; β-carotene/linoleate bleaching	-Insignificant correlation between TAC and TEAC ($r=0.154$), ALPA($r=0.03$), and DPPH value ($r=-0.182$).	[63]
11	Twenty accessions collected from northern Greece	DPPH	-Antioxidant activity was corelated with TAC ($r=0.513$) and ascorbic acid ($r=0.577$), but not TPC.	[64]
12	Five ecotypes of 'Gabsi' variety, and 4 ecotype of 'Tounsi' from Tunisia	ABTS	-A significant correlation was found between the total antioxidant capacity and proanthocyanin contents ($r=0.80$*) but not TAC.	[65]
13	Six ecotypes from Tunisia	FRAP; ORAC,	-In peel, the antioxidant activities significantly correlated with TAC ($r=0.800$* by FRAP, $r=0.898$** by ORAC), hydrolysable tannins ($r=0.788$* by FRAP, $r=0.870$** by ORAC), and gallic acid ($r=0.715$* by FRAP, $r=0.971$** by ORAC). In juice, it was ORAC results but not FRAP correlated significantly with TAC ($r=0.850$**).	[66]
14	Six cultivars from Tunisia	ABTS; DPPH; FRAP; ORAC	-DPPH highly correlated with TFC ($r=-0.960$**), TAC ($r=-0.866$**), and TPC ($r=-0.788$**). FRAP results highly correlated with TFC ($r=0.662$**), TAC ($r=0.693$**). ORAC results highly correlated with TFC ($r=0.763$**), TAC ($r=0.850$**). ABTS values were not correlated with TFC, TAC, and TPC.	[67]
15	Six old Italian pomegranate cultivars	ABTS; DPPH; FRAP	-A significant correlation between juice TPC and antioxidant activity by ABTS ($r^2=0.943$).	[68]
16	Three cultivars 'Arakta', 'Bhagwa', and 'Ruby' from South Africa	DPPH; FRAP; total antioxidant capacity	-Neither TAC nor TFC correlated with antioxidant capacities based on three assays results. A significant correlation was found in TPC, TTC, proanthocyanidins, gallic acid with FRAP or total antioxidant capacity, but not with DPPH results.	[69]
17	Eighteen cultivars from Morocco	ABTS; DPPH; FRAP	-TAC or TPC was not significantly correlated with antioxidant capacities by DPPH, ABTS, and FRAP asays. A high positive correlation values was shown between the flavonoid content and antioxidant capacity ($r=0.931$ DPPH, 0.668 FRAP, 0.660 ABTS).	[70]
18	10 Chinese cultivars from 4 different regions	ABTS; DPPH; SASC; TRC	-Punicalagin concentration in aril juice significantly correlated with the 4 antioxidant capacities ($P<0.01$). None of other detected phenolic monomers was correlated significant with antioxidant capacities.	[71]

Table 3. (cont.)

No.	Samples	Antioxidant assays	Key findings	Reference
19	Juices of 6 cultivars from southern Turkey	FRAP; TEAC	-Levels of FRAP, TEAC, TPC, and total monomeric anthocyanins were strongly correlated (r = 0.82 ~ 0.96).	[72]
20	8 genotypes from Chile	ORAC	-A significant correlation between the ORAC values with TAC (r = 0.89*) and with TPC (r = 0.95*).	[73]
21	29 accessions from Israel	DPPH; FRAP	-Aril juice: TAC significantly correlated to the antioxidant activity by FRAP (r = 0.68**), but insignificantly by DPPH (r = 0.265). Whole fruit homogenates: TPC but not TAC correlated positively with antioxidant activity (r = 0.95** by FRAP, r = 0.71**by DPPH). Fruit peel: TPC correlated to the antioxidant level (r = 0.95** by FRAP, r = 0.55** by DPPH), not correlation was found between antioxidant activity and TAC.	[75]
22	13 cultivars from Southern Tunisia	ABTS; DPPH; FRAP	-TPC but not TAC correlated significantly and positively with the antioxidant capacity of juice.	[76]
23	Fresh ripe pomegranate fruits of 11 cultivars from Israel	FRAP	-TAC correlated highly to the antioxidant levels (r = 0.84**), and to total phenolic content (r = 0.70**).	[81]
24	Fresh ripe pomegranate fruits of 11 cultivars from Israel	FRAP	-The antioxidative capacity was linearly correlated with TPC ($R^2 = 0.98$), but poorly correlated with TAC ($R^2 = 0.38$).	[83]
25	'Mollar' from Spain	ORAC	-ORAC results were strongly correlated with total monomeric anthocyanin content.	[112]
26	Six cultivars form India	CUPRAC; DPPH; FRAP	-There was significant correlation of TPC with antioxidant activity in all assays ($R^2 = 0.83$ FRAP, 0.90 DPPH, 0.96 CUPRAC). TFC was strongly correlated with all assays ($R^2 = 0.75$ FRAP, 0.82 DPPH, 0.85 CUPRAC).	[113]
27	16 fresh cultivars from different regions of Turkey	ABTS; DPPH; FRAP	-A high correlation between antioxidant capacity and TAC ($r = 0.94$), TPC($r = 0.94$) and ascorbic acid($r = 0.75$).	[114]
28	Tounsi and Nana from Tunisia	DPPH; FRAP	-Anthocyanins were the main contributors to antioxidant activity in juice detected by DPPH. -Antioxidant activity was mainly due to the presence of tannins and carotenoids in peel extract.	[115]
29	Fresh fruit of California, standard Cy, Dp, and Pg	Free radical (·OH, $O^{2\cdot-}$, NO) scavenging; Inhibition of lipid peroxidation system	-Pomegranate extract and three standard anthocyanidins directly scavenged $O^{2\cdot-}$in a dose-dependent manner, but indirectly scavenged ·OH. They did not show NO scavenging activity *in vitro*. Inhibition H_2O_2-induced lipid peroxidation in rat brain homogenates was in order of Dp > Cy > Pg.	[100]
30	Flowers from Turkey	DPPH; β-carotene/linoleic acid system; Rancimat Method	-Antioxidant capacity was correlated with the TPC.	[116]

** and * indicate significant at $p < 0.01$ and $p < 0.05$, respectively. **Abbreviations**: ABTS: 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid; AEAC: Ascorbic acid equivalent antioxidant capacity; ALPA: Anti-lipid peroxidative activity; CUPRAC: Cupric ion

Table 3. (cont.)

No.	Samples	Antioxidant assays	Key findings	Reference

reducing antioxidant capacity; DMPD: N,N-dimethyl-p-phenylenediamine; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FIC: Ferrous ion chelating; FRAP: Ferric-reducing antioxidant power; NBT: Nitroblue tetrazolium chloride; ORAC: Oxygen radical absorbance capacity; SASC: Superoxide anion scavenging capacity; TAC: Total anthocyanin content; TEAC: Trolox equivalent antioxidant capacity; TFC: Total flavonoids content; TOSC: Total oxyradical scavenging capacity; TPC: Total phenolic content; TRC: Total reducing capacity; TTC: Total tannin content.

and antioxidant activity measured by DPPH, ABTS or FRAP assays,[22,25,34,38,47,63,68,72,83,113] or high correlation between these detection assays.[68,114] That means, these methods are consistent in total antioxidant capacity measurement. This correlation could be explained by the similar redox reactions that these *in vitro* methods rely on, that is, their mechanisms concerning the ability of the antioxidants to reduce certain radicals (DPPH radical, ferric iron, and ABTS radical). Therefore, It is better to apply different types of assays instead of somewhat redundant assays in quantifying antioxidant capacity.[106]

However, these results must be interpreted with caution as the method determined by the Folin-Ciocalteu overestimates the concentration of phenolics due to the interference of other compounds such as ascorbic acids and vitamins, thus sometimes do not give significant correlation.[96] Some reports have also claimed a poor correlation between TPC and antioxidant activity measured by DPPH, ABTS and FRAP assays in pomegranate.[67,70,117]

According to some reports, anthocyanins in pomegranate showed apparently different reactive oxygen (ROS) and nitrogen species (RNS) scavenging activities.[17] Noda et al. found that standard anthocyanidins and pomegranate extract did not effectively scavenge nitric oxide (NO) radical, but inhibited $\cdot O^{2-}$ and ·OH radicals in a dose-dependent manner. Pomegranate extracts and pure Dp, Cy, and Pg directly scavenged $\cdot O^{2-}$, with Dp was the dominant component; when encountered ·OH, these compounds presented scavenging activity in the ·OH generation system except Pg. The authors inferred that there may be a different oxidation mechanism of Pg in ·OH scavenging activities.[100] An inhibition against H_2O_2-induced oxidative stress was observed in cultured human hepatocellular HepG2 cells when performed with separated anthocyanins and copigment fraction from pomegranate juice,[39] indicating their potential action as cellular ROS scavengers. Inhibitory effects of anthocyanins on H_2O_2-induced lipid peroxidation were determined in rat brain homogenates, with an order of Dp > Cy > Pg, which was in line with the results of radical scavenging activity.[100]

Therefore, it is not surprising that investigators following different approaches often achieve different results, because the antioxidant action is very much dependent on the assay used. Without a unified standard, it is still a challenging task to select the applicable assays and model systems for antioxidant capacity determination.

4.4. The Correlation Between TAC and Antioxidant Capacities of Pomegranate

In several previous *in vitro* studies, correlation analyses have been performed to aid understanding of the antioxidant role of anthocyanins. A variety of findings support high antioxidant power of anthocyanins in pomegranate aril juice, whole-fruit juice, peel, and flower. According to the results indicated in *Table 3*, some authors believed that the antioxidant activity in aril juice correlated significantly to TAC,[24,37,72,73,75] suggesting that anthocyanins play a key role in antioxidant potential in aril juice. According to the results of β-carotene bleaching method, the acidity and anthocyanins were both major contributors to the *in vitro* antioxidant activity in aril juice and peel.[58] More recently, Kostka et al. revealed no significant differences between the anthocyanin and polyphenol of pomegranate towards radical scavenging activity against DPPH and galvinoxyl radicals, thus suggesting equal importance of the anthocyanins and polyphenols in terms of antioxidant capacities in pomegranate juices.[39]

However, other studies reported that there was no correlation between anthocyanin levels and the antioxidant capacity of fruit juice.[40,63,69,76,83] It was believed that antioxidant activity of pomegranate juice was better correlated with total phenolics than anthocyanins independent of *in vitro* assays used,[22,25,34,76,83,113] supporting the view that phenolics were the principal contributors to the high antioxidant activity measured in pomegranate juice. Of the

phenolics compounds, some authors believed that punicalagin was one of the most contributed antioxidants,[71] while others denied the significant correlations between total punicalagins and antioxidant activities.[34] Other findings suggested the importance of phenolic acids, ascorbic acids, and flavonoids in the total antioxidant activity of pomegranate.[33,113] Conversely, El Kar et al. believed that the antioxidant activity was not significantly correlated to TPC, but significantly related to total proanthocyanins content in juices.[65] A high correlation between total antioxidant capacity and TPC, TAC, as well as ascorbic acid was observed by Karav et al.,[114] but another study revealed that it was TAC and ascorbic acid, but not TPC significantly correlated with antioxidant activity.[64] From these above-mentioned results it could be preliminarily concluded that anthocyanins were not the only major contributors to the overall antioxidative capacity exhibited by juice.

For the antioxidant potential of commercial pomegranate juices or whole-fruit squeezed juices, there seems to be less controversial findings among different authors. Tzulker et al.[75] and Gil et al.[60] pointed out that the antioxidant activity of the whole fruit homogenates was much higher than that found in aril juice. The presence of phytochemicals such as hydrolyzable tannins and organic acids in pomegranate peels of the whole fruit preparations may be responsible for the higher antioxidant properties of whole-fruit juices. This was confirmed by Tzulker et al., who reported that the antioxidant potentials from the whole fruit homogenates correlated significantly with the content of hydrolyzable tannins, but not correlated with the concentration of anthocyanins.[75] The much higher antioxidant capacities in whole-fruit squeezed juices indicated that the peel was a much more efficient antioxidant than juice.[58,75] In fruit peel, hydrolyzable tannins together with anthocyanins,[66] or carotenoids,[24] or total flavonoids and O-diphenols[62] contributed significantly to the antioxidant activities. This fact proved conclusively that it was hydrolyzable tannins, not anthocyanins, played a lead role in the antioxidant potential of fruit peel, because no correlations were found between antioxidant activities and the anthocyanin levels.[58,62,75] In addition, the anthocyanins in flowers also exhibited considerable antioxidant potentials.[10,27,116] The antioxidant capacity of the flower was correlated with the TPC[116] but not with TAC.[62] However, anthocyanins, both Pg3G and Pg3,5dG showed strong antioxidant capacities.[10] Altogether, it can be concluded that anthocyanins play an indispensable role in the antioxidant function of the pomegranate, though there are some exceptions among the detection results.

5. Conclusion and Future Scopes

It is obvious that pomegranate is characterized by high genetic diversity of anthocyanin traits. Pomegranate, like other fruits, varies in anthocyanin composition, even within the same cultivar, depending on plant parts, maturity, location, as well as numerous other environmental factors. The predominant anthocyanins present in pomegranate are delphinidin 3,5-di (Dp3,5dG) and 3-O-glucoside (Dp3G), cyanidin 3,5-di (Cy3,5dG) and 3-O-glucoside (Cy3G), pelargonidin 3,5-di (Pg3,5dG) and 3-O-glucoside (Pg3G). Mono- and di-glucosylated Dp and Cy occur as the prominent anthocyanins and pelargonidins as minor components. An accepted view is that anthocyanins are abundant in fruit juice, while hydrolyzable tannins are rich in fruit peel. As primary antioxidants, anthocyanins inactivate free radicals via hydrogen-transfer reactions from the phenolic OH group to radicals. Metal chelating activities exerted by anthocyanins are hardly conclusive because either negligible or considerable values are registered in pomegranate. Basically, antioxidant activity of anthocyanins is strictly dependent on their chemical structure involving the number and position of OH groups in the pyrone-ring. The different antioxidant action shown by anthocyanins depends partly on the assays used. Anthocyanins, together with other polyphenols especially hydrolyzable tannins, contribute to the overall antioxidant capacities of pomegranate fruits and flowers.

Nonetheless, the anthocyanin antioxidative puzzle in pomegranate is not solved yet. Several key issues need to be resolved before a comprehensive understanding of the antioxidant effects of anthocyanins in pomegranate. Firstly, the interrelation between the chemical structure of anthocyanin molecules and their antioxidant activities remains largely unclear. Thus, it is crucial to elucidate which type of anthocyanin species exhibits higher antioxidant capacities. Secondly, it is certain that total anthocyanin alone cannot explain the antioxidant activity of pomegranate since it is proposed that the synergistic or additive action of anthocyanins together with other phenols in fruits accounts for their superior antioxidant properties. There is a need for further mechanistic investigations focused on antioxidant interactions between anthocyanins and other polyphenols. The last but not the least, the working mechanisms of antioxidant systems

require the detailed study of their reactions with the biological molecular targets. Hence, it is essential to perform *in vivo* tests in order to give a comprehensive prediction of the antioxidant efficiency of pomegranate anthocyanins after screening antioxidant activity with *in vitro* methods.

It is irrefutable that the exploitation and utilization of plant-derived natural antioxidant is a rapidly emerging trend around the world. Anthocyanins from the peel, aril juice, flowers make pomegranate a promising ingredient in the development of nutraceutical and functional foods. All this will further facilitate understanding the anthocyanin profiles in pomegranate, and provide a better insight into the antioxidant properties of pomegranate anthocyanins.

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Author Contribution Statement

X.-Q.Z. wrote the article. Z.-H.Y. reviewed and modified the manuscript.

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