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Bright Side of Bilberry: Exploring It's Impact On Age Related Macular Degeneration

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Abstract

Age-related macular degeneration, often called AMD or ARMD, is the leading cause of vision loss and blindness. It usually affects the older population mainly those who are 60 years and older. It is the degeneration of the macula, which is the part of the retina responsible for the sharp, central vision needed to read or drive. Because the macula primarily is affected by AMD, central vision loss may occur. There are mainly two types of AMD the Wet form and the Dry form. The dry form is more common than the wet form, with about 85 to 90 percent of AMD patients diagnosed with dry AMD. The wet form of the disease usually leads to more serious vision loss.
Anthocyanin-rich bilberry, a plant-derived antioxidant, has been utilized as a popular supplement for ocular health worldwide. In addition to its anti-inflammatory effect, the anthocyanin-rich bilberry extract ameliorated the intracellular elevation of reactive oxygen species and activated NF-κB, a redox-sensitive transcription factor, in the inflamed retina. Because of these properties, bilberry can be a complementary treatment for those who are looking for the treatment of AMD.

1. Introduction:

Changes associated with age impact every aspect of the eye. While these changes can impact how well we see, it's important to distinguish between natural age-related shifts and abnormal changes. Age-related macular degeneration (AMD or ARMD) is a medical condition that can lead to blurry or absent central vision. This condition usually appears in older individuals. Visual impairment becomes more common after the age of 60, making it the second most prevalent physical limitation in the elderly. Genetics and smoking are contributing factors. AMD occurs due to harm to the central part of the retina, known as the macula. Detection involves a comprehensive eye examination, and its severity is categorized as early, intermediate, or late stages. [(1)]. The late type is additionally divided into "dry" and "wet" forms with the dry form predominantly being 90% of cases [(2)] During the year 2020, approximately 196 million instances of age-related macular degeneration were estimated globally. This figure is predicted to ascend to 288 million by the year 2040. Projections pointed towards Asia harboring the largest number of cases by 2040 (around 113 million), succeeded by Europe (approximately 69 million), Africa (about 39 million), as well as Latin America along with the Caribbean (roughly 39 million). Additionally, North America was expected to have around 25 million cases, whereas

Oceania would have a smaller count of 2 million. Upon analyzing statistical contrasts, it was evident that Asia's count would likely surpass that of other regions by 2040. This trend was anticipated to persist until roughly 2038 for the African region.[3] Aging leads to visible yellow deposits called drusen in the retina, positioned between the retinal pigment epithelium and Bruch's membrane. Age-related macular degeneration (AMD) patients exhibit diverse clinical features based on drusen size and pigment irregularities, including both lighter and darker pigmentation changes. [2] Situated at the rear of the eye, the macula holds light-responsive cells that facilitate sharp central eyesight. This component is vital within the retina, transforming light into electrical impulses which are relayed through the optic nerve to the brain, forming images. The degeneration of the macula is a gradual deterioration process, which could potentially give rise to macular gaps. Though these gaps generally do not stem from injuries, forceful impacts have the potential to damage the macula's vascular system, ultimately causing its deterioration.. [1]

Bilberry (Vaccinium myrtillus L.) emerges as a natural source of Anthocyanins, a cluster of polyphenolic compounds accountable for its vivid blue/black hue and substantial antioxidant content. Renowned for enhancing eyesight, bilberry presents a spectrum of potential health benefits. Research indicates that bilberry might contribute to reducing blood sugar levels, displaying anti-inflammatory characteristics, and aiding in the reduction of lipid levels. Moreover, its antioxidative traits foster robust protection against oxidative tension. Given these attributes, bilberry holds promising potential in preventing and managing various conditions linked to inflammation, dyslipidemia, elevated blood sugar, oxidative strain, and age-related illnesses. [4]

Oxidative harm, inflammation, and the growth of new blood vessels (neovascularization) play pivotal roles in the development of AMD. Consequently, antioxidants like dynamic constituents, carotenoids, extracts, polysaccharides, flavonoids, formulations, vitamins, and whole foods could possess the capability to hinder or postpone the advancement of the disease. We will delve into the documented molecular methods through which bilberries operate, encompassing their safeguarding mechanisms. This assessment furnishes a systematic foundation concerning the application of antioxidants as remedies for AMD, spanning from laboratory experiments to clinical tests.

2. Classification and severity grading of AMD:

AMD has two main types of macular degeneration known as dry and wet AMD. Dry AMD, which is also known as the nonexudative form, is the most common type, comprising ~90% of all diagnosed cases. GA is the advanced stage of dry AMD. Wet AMD is also known as the exudative form, and although less common than dry AMD, it is associated with a more rapid progression to advanced vision loss. The main manifestations of wet AMD are CNV and pigment epithelial detachment (PED). Approximately 10%–20% of patients with nonexudative AMD may develop the wet form, which is estimated to affect 1.75 million people in the US.

The development of standardized photographic retinal grading methods, like the Wisconsin ARM grading system, was followed by the International ARM Epidemiological Study. This study redefined the diagnostic system for Age-related Macular Degeneration (AMD) and implemented a stricter criterion for its diagnosis. The ARM grading system characterized minimal or moderate nonexudative age-related changes in the macula. To establish a diagnosis of nonexudative AMD or wet AMD, the presence of advanced RPE atrophy (geographic atrophy - GA) or choroidal neovascularization (CNV) respectively, was essential. After applying the International Classification criteria, ARM accounted for approximately 85%–90% of AMD cases, while nonexudative AMD represented 10%–15% of affected individuals with age-related macular changes-.[5]

The Age-Related Eye Disease Study (AREDS) further defined categories of AMD based on the presenting features of drusen (deposits under the retina), atrophy, and neovascularization. These categories are as follows:

1. No AMD: Fewer than five small drusen.
2. Early AMD: Multiple small drusen or at least one intermediate-sized drusen.
3. Intermediate AMD: Extensive intermediate-sized drusen, more than one large drusen, or non-central GA.
4. Late AMD: Central GA or CNV causing vision loss (visual acuity worse than 20/32) in one eye. it is further devided into Dry AMD and Wet AMD. [6]

Dry AMD:

Drusen are one of the earliest signs in AMD. Clinically, typical drusen appear as focal, whitish yellow excrescences deep to the retina. Typical drusen deposits are located beneath the retinal pigment epithelium and Bruch’s membrane and vary widely in number, shape size, and distribution. Most drusen are 20-100 µm and are characterized as hard or soft. Hard drusen, which appear as round, discrete yellow-white spots are commonly identified in many populations. They are not age-related and do not carry an increased risk for the

development of neovascularization[8,9]. On the other hand, soft drusen are characterized by their vague boundaries and lack of distinct edges, with a size equal to or exceeding 63 µm. Various research endeavors and experiments have consistently shown that these larger, merged soft drusen are closely linked to age-related changes and are connected to an elevated likelihood of advanced AMD onset, often accompanied by the growth of abnormal blood vessels (neovascularization)[9,10]. Clinically, geographic atrophy stands out due to its distinct characteristics. It presents as a clearly defined region within the retina where the thickness is reduced compared to the surrounding area. This variance in thickness results in a noticeable change in color, making the choroidal vessels underneath more visible. Alterations in pigmentation, such as lighter or darker areas, can encircle the macular atrophy. When the central point of the retina (foveal center) remains unaffected, there's a chance that visual acuity will remain decent. However, reading vision might still be compromised due to a narrowed central visual field[11].

Wet AMD:

Wet AMD is distinguished by the occurrence of new vessel growth within the macula. Choroidal neovascularization (CNV) refers to the emergence of new blood vessels from the choriocapillaris, which breach the outer part of Bruch's membrane and extend into the space beneath the pigmented epithelium. Clinical indications of neovascular AMD encompass various aspects: accumulation of fluid beneath the retina or within it, bleeding in the retina, subretinal region, or beneath the retinal pigment epithelium (RPE), discharge of lipid deposits, as well as the presence of gray or yellow-green discoloration or membrane-like formations. RPE detachment and tearing can also be observed. In the advanced stage of the disease, the neovascularization leads to the formation of a scar composed of fibrous and vascular tissue (disciform scar) or results in atrophy. This eventually causes lasting harm to central vision. [12,13,14] Pigment epithelial detachment (PED) is a separation of the retinal pigment epithelium (RPE) that can arise from factors like serous fluid, fibrovascular tissue, hemorrhage, or the merging of drusen beneath the RPE layer. A serous PED presents as a convex detachment of the RPE, displaying prominent and widespread brightness in fluorescein imaging. Over time, there's a gradual accumulation of fluid in a confined area, leading to pooling. [15]

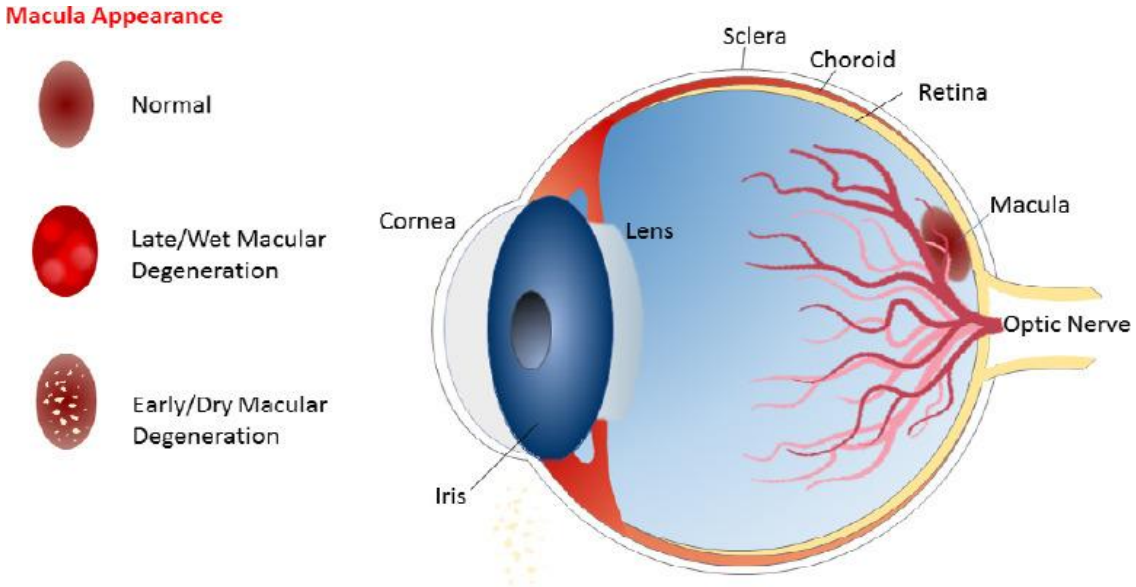


Figure 1: Age-related macular degeneration types and macular appearance [16]

3. Risk Factors:

Age:

Advanced AMD development has various risk factors including age, ethnicity, and genetics. Age is the primary predictor, with the risk increasing over threefold in those over 75 compared to ages 65-74. In the US, around 30% of individuals above 85 years have AMD. [17,18]

Family history and genetics:

AMD prevalence varies among ethnic groups, with Caucasians having the highest rates, followed by Hispanics and Asians, and the lowest rates in African Americans. Family history significantly increases AMD risk,

particularly among siblings. Genetic factors play a significant role in AMD, with 34 genetic loci and 52 gene variants identified. These genes affect immune response, inflammation, and retina health. Notable genes include CFH, HTRA1 (ARMS2), and CFB/C2. Genetic makeup also influences treatment response, impacting age of onset and response to anti-VEGF therapies. Incorporating genetics into treatment strategies could enhance personalized care for AMD patients. Studies highlight cumulative gene effects on disease onset and treatment response, suggesting potential for tailored therapeutic approaches based on individual genetic profiles.[5]

Lifestyle, diet, and nutrition:
The primary modifiable risk factor for AMD is smoking, and patients should be consistently advised to quit smoking during their visits to prevent worsening vision loss. Individuals who have smoked for over 40 years face a two to fourfold higher likelihood of AMD compared to non-smokers of similar age [18]. The ALIENOR study from France showed that high pulse pressure was associated with an increased risk of late-stage AMD, whereas systolic or diastolic blood pressure or the use of antihypertensive medications was not significantly associated with an increased risk of either early- or late-stage AMD. Consuming saturated fats, trans fats, and omega-6 fatty acids is linked to a twofold rise in AMD prevalence, while monounsaturated fats might offer a protective effect. [19]

4. Antioxidant activity of Bilberry :

The berries found in various species of Vaccinium are notably rich in antioxidant compounds, making them a valuable dietary source for supplementing antioxidants. V. myrtillus, in particular, is distinguished by its content of delphinidin and cyanidin glycosides, as well as quercetin and chlorogenic acid.[19] While investigating the antioxidant properties of the complete extract, a significant synergistic effect was observed. The majority of observed effects were linked not to the isolated anthocyanin fractions, but rather to the unseparated extract as a whole. [21]

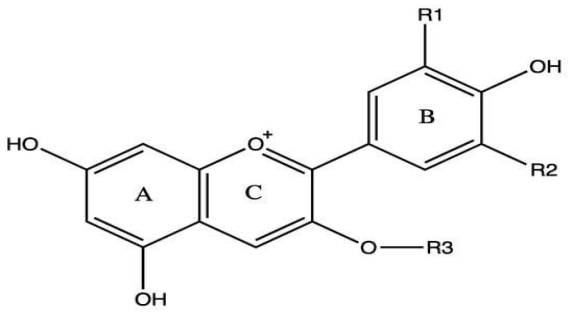
Recent studies have suggested that anthocyanins and other polyphenols can also have indirect effects by stimulating antioxidative defence mechanisms via induction of enzymes such as GST or GSH-Px [21] or DAF-16 and HSF-1 [22] Typically, these mechanisms exhibit dependence on the dosage. Nevertheless, an examination involving isolated rat hearts revealed that elevated concentrations of bilberry anthocyanins (ranging from 5 to 50 mg/L) might lead to reduced cardioprotective effects and demonstrate potential harm to the heart, despite the fact that their ability to scavenge radicals and function as intracellular antioxidants increased proportionally with the concentration. [23]

Antioxidant activity plays a crucial role in facilitating numerous positive effects within the human body. Extracts from bilberries, for instance, hinder the oxidative alteration of human LDL (low-density lipoprotein) in laboratory settingsn attenuate liver damage induced by CCl4 or stress in rats[24]. ameliorate oxidative stress after ischaemia-reperfusion injury in isolated rat heart[23]. during metabolic syndrome[26] or in cancer cells[24].

5. Bioactive Constituents of Bilberry:

Anthocyanin:
The fruits of each above listed species have a characteristic anthocyanin profile which can be used for fingerprint analysis:

- V. myrtillus contains mainly delphinidin and cyanidin (Figure 1) in a 1 : 1 ratio, followed by petunidin, peonidin and malvidin
- V. vitis-idaea contains mainly cyanidin (>90%) and small amounts of peonidin, other anthocyanins are absent
- V. × intermedium typically contains more cyanidin compared to bilberry
- V. corymbosum contains mainly delphinidin, peonidin is absent
- V. uliginosum contains delphinidin and malvidin in 1 : 1 ratio (figure 1)
- V. myrtillus has the most intensely colored berries out of all above mentioned species. This is due to the fact that both peel and pulp contain large amount of anthocyanidins (up to 2% of the fresh weight in peels), whereas in other species the pulp is white or light pink at most [28] . In bilberries, anthocyanins comprise about 90% of the total phenolic compounds of the fruit [29]



Anthocyanin (% in Content in Bilberry)	R1	R2	λ_{max} (nm) *	
			R3=H	R3=gluc
Delphinidin (15.17%)	OH	OH	546	541
Cyanidin (8.36%)	OH	H	535	530
Petunidin (6.64%)	OH	OCH_3	543	540
Malvidin (5.43%)	OCH_3	OCH_3	542	538
Peonidin (1.87%)	OCH_3	H	532	528
* In methanol with 0.01% HCl				

Figure 2. Structures of the main anthocyanin-3-O-glucosides found in bilberry and respective wavelength at the maximum absorption in the visible region (λmax). [30]

Flavonols:

Quercetin is the main flavonol of bilberry fruits, accounting for more than 50% of total flavonoid content[27] . The second most abundant one is myricetin (Figure 1); other flavonols, such as syringetin, laricitrin and isorhamnetin, have only been detected in low levels Kaempferol, although abundant in bilberry leaves, is present in fruits only in trace amounts. Flavonols in V. myrtillus occur mainly in glycosylated form. Studies determined various hexosides, pentosides and glucuronides. The most abundant glycosides appear to be rhamnosides and glucuronides. Phlorizin, a glucoside of a dihydrochalcone phloretin, was found in bilberry fruits, however the aglycone itself was not detected [27] .

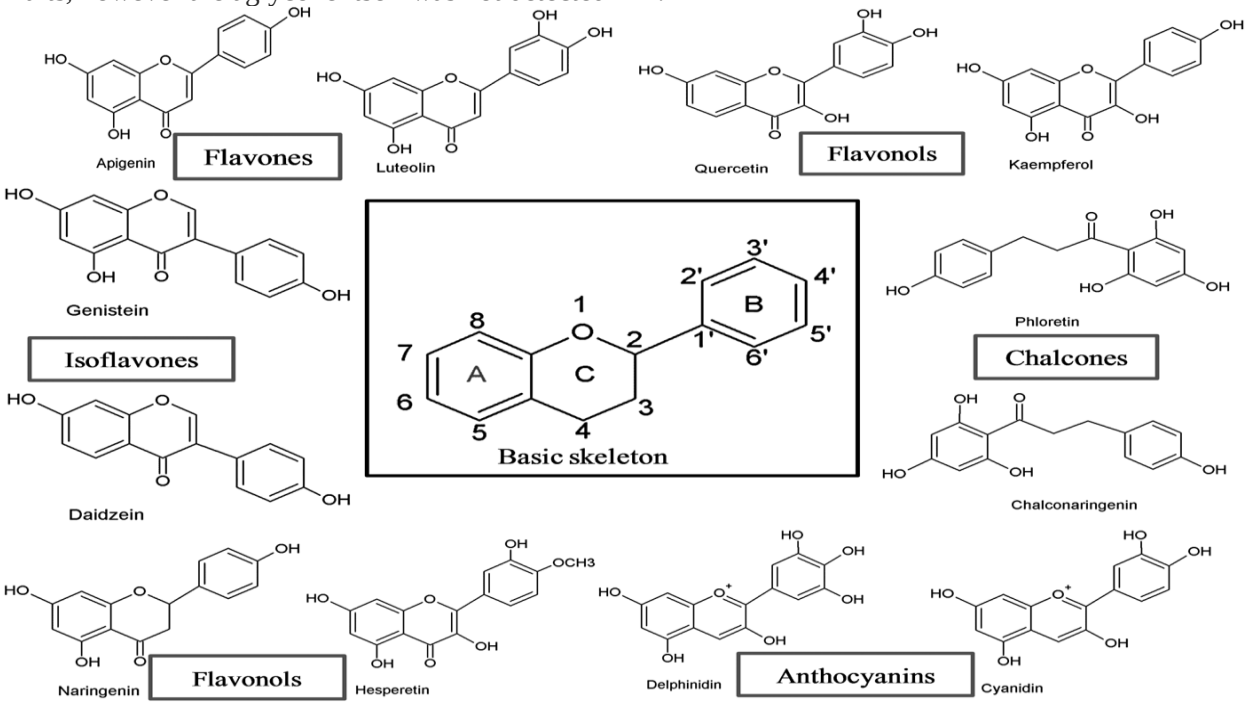


Figure 3 : Basic skeleton structure of flavonoids and their classes. [31]

Triterpenoids:

The whitish wax cuticle covering the bilberry fruits consists mainly of triterpenoids such as α- and ß-amyrin, oleanolic and ursolic acid (Figure 2), as well as fatty cerotic acid, sitosterol and 2-heneicosanone. The wax layer of bilberries is similar to that of blueberries and much thinner than that of lingonberries and crowberries[32]. Oleanolic and ursolic acid, α- and ß-amyrin, lupeol, lupenyl acetate and betulin were identified in the extract of bilberry leaves[27].

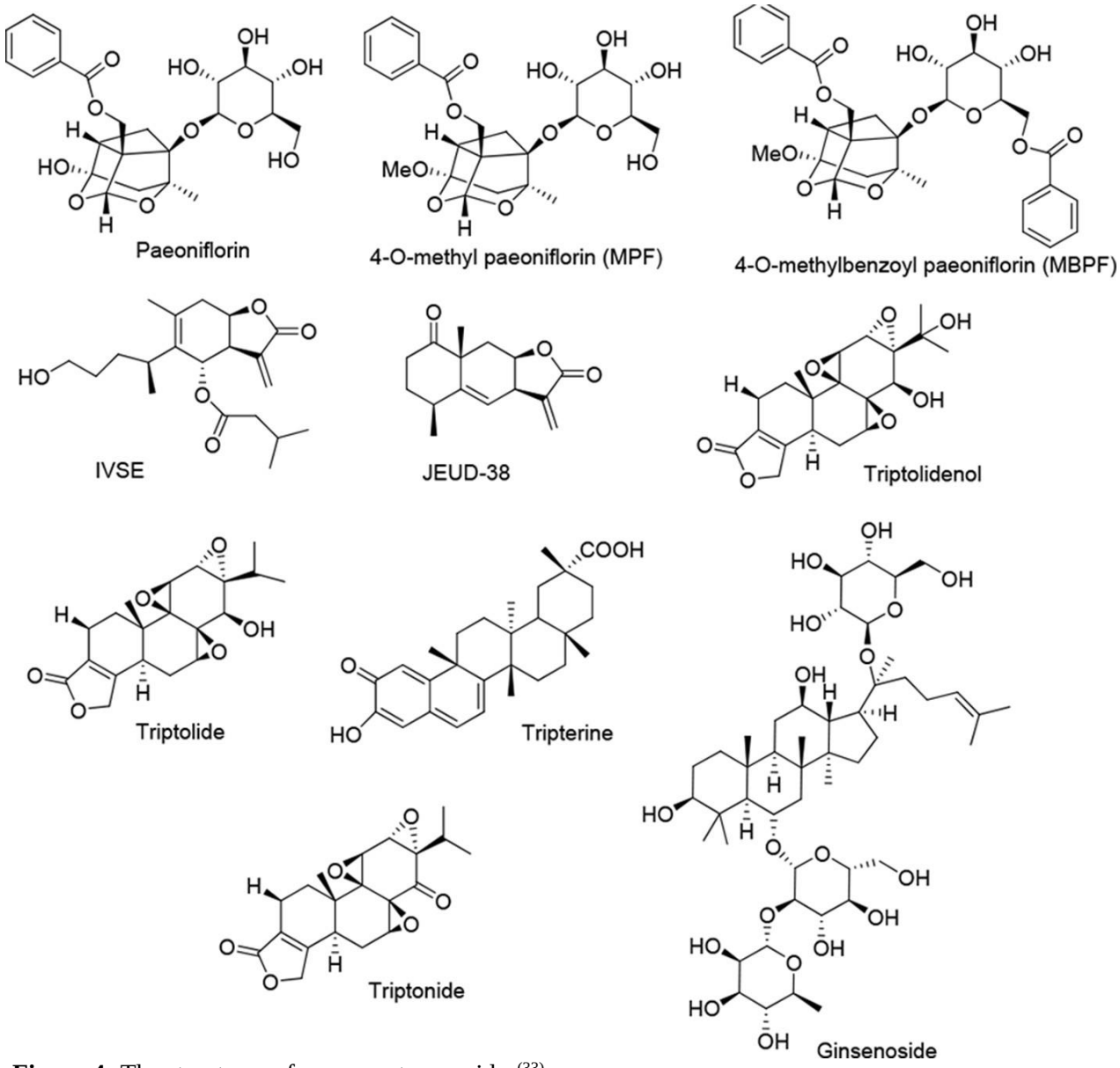


Figure 4: The structures of common terpenoids [33]

6. Presence of Oxidative Stress Markers in Clinical AMD:

As oxidative stress is one of the major factors contributing to and worsening the pathological events occurring in AMD, many physiological conditions unique to the retina environment facilitate ROS generation and oxidative stress. These include high polyunsaturated fatty acid content in the photoreceptors for lipid peroxidation, high oxygen demand and mitochondrial metabolism, extensive light exposure to the retina, and the presence of lipofuscin.[34] In dry AMD characterized by RPE degeneration in late disease progression, the RPE provides protection to the retina against light-induced oxidative damage by absorbing excess photons. The RPE also has a high oxygen consumption rate from their high mitochondrial oxidative metabolism. RPE cells are hence highly susceptible to oxidative stress.[35] Although retinal pigment epithelium (RPE) cells possess intrinsic antioxidative mechanisms to defend against oxidative stress, the aging process can compromise the efficiency of RPE's antioxidative machinery, rendering it less capable of effectively

countering oxidative harm.[36,37] Oxidative stress-induced RPE damage thus plays an important role in the progression of dry AMD.[37]

Compelling evidence validating the existence of biomarkers for oxidative stress in age-related macular degeneration (AMD) has been derived from clinical investigations involving post-mortem donor eyes. Notably, oxidative DNA damage, including the presence of 8-hydroxy-2-deoxyguanosine (8-OHdG), was observed in AMD donor eyes, particularly in cases of dry AMD accompanied by retinal pigment epithelium (RPE) atrophy.[38] Since the photoreceptors' outer segments are lipid enriched with docosahexaenoic acid (DHA)[39] enhanced levels of carboxyethylpyrrole (CEP) protein adducts, a biomarker of DHA-containing lipid peroxidation, in the BM of AMD donor eyes also indicate high vulnerabilities toward oxidative stress in AMD eyes compared to non-AMD.[40,41,42] In addition, increased CEP levels (~60% elevation) were detected in ELISA in dry AMD eyes compared to control donor eyes.[43,44]

Beyond nuclear DNA oxidative damage, AMD donor eyes also showed RPE mitochondrial DNA damage due to oxidative stress.[45,46] suggesting that an imbalance in RPE mitochondrial homeostasis and metabolic dysfunction due to oxidative stress may also lead to macular degeneration in AMD.[47] To this end, mitochondrial defects in RPE cells are being recognized as one of the major features in AMD pathology. This notion is supported by studies of mitochondrial and glycolytic functions in RPE, which were reduced in RPE cells isolated and cultured from AMD donor eyes compared with non-AMD eyes with AMD-derived RPE cells showing lower levels of glutathione and ATP. Moreover, significantly higher levels of peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) protein in these cells as compared to healthy RPE may also reflect susceptibility and compensatory response toward oxidative stress. [48] PGC-1α has beneficial and protective effects on mitochondrial metabolism and antioxidant capacity[49,50,51], which might enhance resistance to and protection against oxidative stress. Another compensatory mechanism of RPE cells to protect against oxidative stress is elevated levels of antioxidant enzymes that scavenge for ROS. Antioxidant enzymes such as catalase, copper- and zinc-containing SOD (CuZnSOD or SOD1), and manganese superoxide dismutase (MnSOD or SOD2) were found to be upregulated in immunoblots of AMD donor eyes in the early and intermediate stages of AMD, which may reflect a compensatory mechanism of upregulating pro-survival signaling to counter increased ROS production in AMD eyes.[50-54]

7. Benefits of Antioxidant Intake in AMD Are Endorsed by AREDS I and II :

Contemporary treatments for wet AMD encompass anti-VEGF therapies, demonstrating notable efficacy albeit with certain constraints. Concurrently, the treatment landscape for dry AMD has seen a recent FDA approval of anti-complement therapy, presenting a modest shield against geographic atrophy (GA), the sole available treatment for this form of AMD as of the present review. Over the preceding decades, two extensive clinical investigations were conducted under the aegis of the National Eye Institute: the Age-Related Eye Disease Study (AREDS) and its sequel, AREDS2. These studies were initiated to probe AMD risk factors and the impacts of antioxidant interventions on AMD progression[35,36]. Dietary intake of antioxidants for slowing AMD progression was supported by findings from the AREDS and AREDS2 studies for slowing AMD progression. Additional studies support that AMD can be partially prevented or that progression can be slowed by several antioxidant molecules and antioxidant-related mechanisms to scavenge excess ROS and to protect against oxidative damage. antioxidants decrease the likelihood of progression of AMD[37] ,as supported by AREDS studies. Conducted with a cohort of 3640 participants, the initial AREDS investigation revealed noteworthy findings. Notably, the utilization of antioxidants, either independently or in tandem, exhibited a substantial reduction in the likelihood of transitioning to advanced stages of AMD.[35] these are the key AREDS ingredient, is a crucial component of many enzymes in maintaining retinal health and metabolism [34]. In AREDS2, a formulation was improved by replacing zeaxanthin with anthocyanin and by reducing zinc, which can further decrease the risk of AMD progression[35,36]. Lutein and zeaxanthin are enriched in the macula region of the retina and are known components of macular pigment. They play important roles in minimizing the oxidative damage and in decreasing the risk of AMD[34]. Although studies have supported the efficacy and beneficial impacts of these antioxidants , their formulations are not universally endorsed, as they have their own pros and cons, which should be carefully considered, particularly in terms of potential interactions with patient genotypes[38]. Overall, these antioxidant supplements have shown great promise to protect against AMD progression (Figure 3)

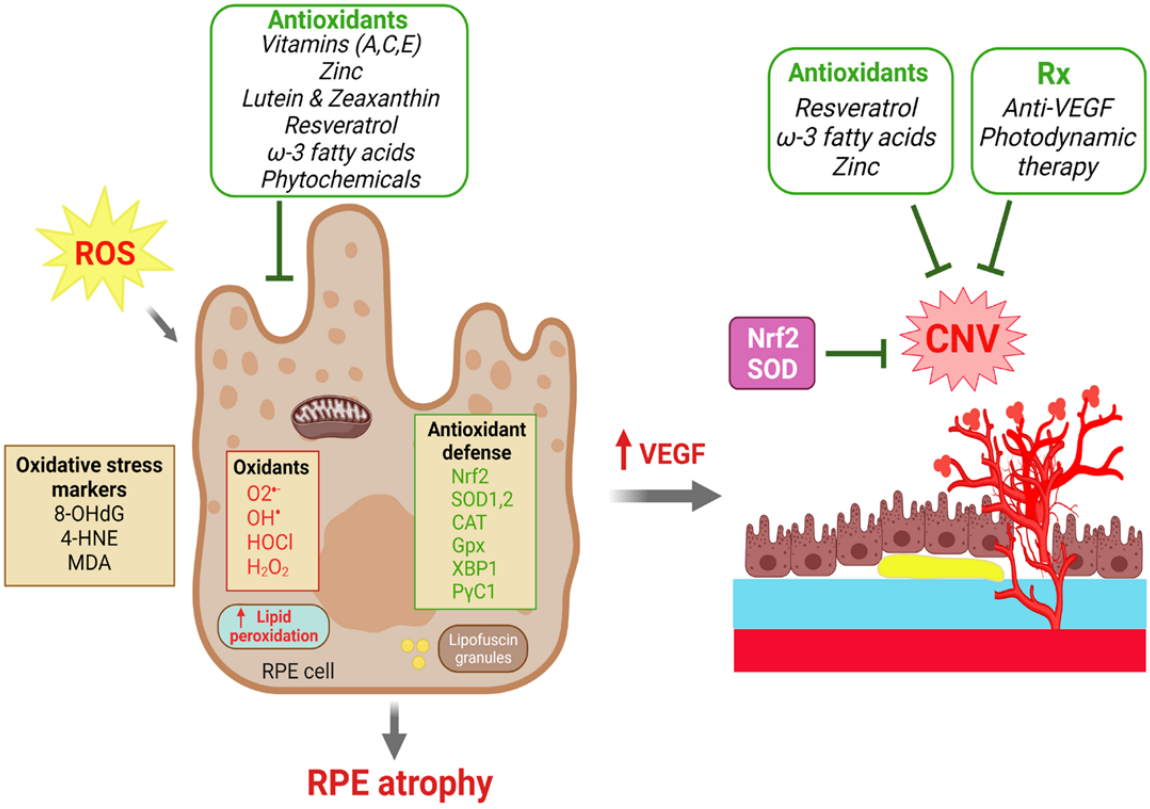


Figure 5. Roles of dietary antioxidants and antioxidant defense in protecting against RPE degeneration and choroidal neovascularization (CNV) in AMD. Excessive reactive oxygen species (ROS) and oxidants cause oxidative damage in the RPE as reflected by increases in oxidative stress markers and lipid peroxidation that eventually leads to RPE atrophy. Upward pointing red arrows indicate a resultant upregulation of the indicated biomolecule. RPE dysfunction and increased VEGF production further lead to CNV formation. The effects of ROS are countered intrinsically by antioxidant reparative defense systems with antioxidant enzymes to scavenger ROS. Multiple dietary antioxidant supplements have been investigated in both clinical trials and experimental studies that have demonstrated protective effects to prevent RPE atrophy and to slow the progression of AMD. CNV formation can be treated by anti-VEGF and photodynamic therapies. Therapeutic strategies against oxidative stress by targeting Nrf2, SOD, catalase and other antioxidants may have protective effects on both RPE degeneration and CNV.[27]

8. Health benefits of bilberry:

- **Nutritional Boost**

100 g of bilberries will give:
Calories: 42
Carbs: 11.5 g (8.7 g sugars and 2.8 g fiber)
Proteins: 0.7 g
Fats: 0.5 g
Vitamin C: 44 mg (48% of recommended daily intake)
Vitamin E: 2.1 mg (14% of recommended daily intake)
Manganese: 3.3 mg (66% of recommended daily intake)
Potassium: 103 mg (3% of recommended daily intake)
This means that bilberries combine a low-calorie count with a high vitamin and mineral content. They're especially rich in vitamin C and manganese.[55]

- **Antioxidant Activity**

The main active components of bilberries are their anthocyanins, powerful antioxidants. These plant pigments also give bilberries their vivid red-to-blue color [4].Bilberries are among the richest anthocyanin sources, supplying 300-700 mg per 100 g berries. The main ones are:
Cyanidin
Delphinidin

Malvidin
Peonidin
Petunidin[56]

- **Eye Health**

According to folk wisdom, bilberries improve eye health and protect against multiple conditions such as glaucoma, cataracts, and eye fatigue.
An extract with bilberry anthocyanins improved vision in a clinical trial on over 300 people with glaucoma. Two similar extracts (standardized to 36% anthocyanins) improved blood retinal flow and reduced eye pressure in 4 trials on over 350 healthy people. These studies suggest that bilberry may, indeed, both improve and prevent glaucoma[57,58].

9. Bilberry Side Effects & Safety

Bilberry is considered safe since people used it as a staple food for millennia. Indeed, a megadose of bilberry anthocyanins (180 mg/kg per day) for 6 months had no toxic effects Of all the clinical trials with bilberry extracts, only 2 reported the following mild adverse effects.
Nausea
Gas
Hard stools
Dying of the tongue and stools
Mild heartburn[59].

10. Conclusion:

Bilberry is having the ability to improve oxygen and blood delivery to the eye, and their potent antioxidant and free radical scavenging properties, suggest a potential benefit for AMD. This protective function was probably caused by the ability of bilberry anthocyanins to increase the antioxidant defense mechanisms, suppress proinflammatory cytokines, and inhibit retinal cells apoptosis. Therefore, bilberry anthocyanins promising candidates as nutritional supplements for the prevention and inhibition of the progression of AMD.

11. References :

1. Gopalasamy K, Gayathri R, Priya VV. Age related macular degeneration: A systematic review. Journal of pharmaceutical sciences and research. 2016 Jun 1;8(6):416.
2. Ratnapriya R, Chew EY. Age-related macular degeneration—clinical review and genetics update. Clinical genetics. 2013 Aug;84(2):160-6.
3. Wong, W.L.; Su, X.; Li, X.; Cheung, C.M.G.; Klein, R.; Cheng, C.Y.; Wong, T.Y. Global Prevalence of Age-Related Macular Degeneration and Disease Burden Projection For 2020 and 2040: A Systematic Review and Meta-analysis. Lancet Glob. Health 2014, 2, e106–e116. [Google Scholar] [CrossRef][Green Version]
4. Chu WK, Cheung SC, Lau RA, Benzie IF. Bilberry (vaccinium myrtillus L.). Herbal Medicine. 2011 Mar 28;20115386:55-71.
5. Al-Zamil WM, Yassin SA. Recent developments in age-related macular degeneration: a review. Clinical interventions in aging. 2017 Aug 22:1313-30.
6. A randomized, Placebo-Controlled, clinical trial of High-Dose supplementation with vitamins C and E, beta carotene, and zinc for Age-Related macular degeneration and vision loss. (2001). Archives of Ophthalmology, 119(10), 1417. https://doi.org/10.1001/archopht.119.10.1417
7. NIH study provides clarity on supplements for protection against. (2015, September 11). National Institutes of Health (NIH). https://www.nih.gov/news-events/news-releases/nih-study-provides-clarity-supplements-protection-against-blinding-eye-disease
8. Martin D, Maguire M, Fine S, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration (AMD) N Engl J Med. 2011;364:1897–1908. [PMC free article] [PubMed] [Google Scholar] [Ref list]
9. Klein R, Klein BE, Knudtson MD, et al. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. Ophthalmology. 2007;114:253–262. [PubMed] [Google Scholar]

10. Cohen SY, Dubois L, Tadayoni R, Delahaye-Mazza C, Debibie C, Quentel G. Prevalence of reticular pseudodrusen in age-related macular degeneration with newly diagnosed choroidal neovascularisation. British journal of ophthalmology. 2007 Mar 1;91(3):354-9.
11. Gheorghe A, Mahdi L, Musat O. Age-related macular degeneration. Romanian journal of ophthalmology. 2015 Apr;59(2):74.
12. Zayit-Soudry S, Moroz I, Loewenstein A. Retinal pigment epithelial detachment. Survey of ophthalmology. 2007 May 1;52(3):227-43.
13. Gregori G, Wang F, Rosenfeld PJ, Yehoshua Z, Gregori NZ, Lujan BJ, Puliafito CA, Feuer WJ. Spectral domain optical coherence tomography imaging of drusen in nonexudative age-related macular degeneration. Ophthalmology. 2011 Jul 1;118(7):1373-9.
14. Chen Y, Vuong LN, Liu J, Ho J, Srinivasan VJ, Gorczynska I, Witkin AJ, Duker JS, Schuman J, Fujimoto JG. Three-dimensional ultrahigh resolution optical coherence tomography imaging of age-related macular degeneration. Optics express. 2009 Mar 2;17(5):4046-60.
15. Holz FG, Bellman C, Staudt S, Schütt F, Völcker HE. Fundus autofluorescence and development of geographic atrophy in age-related macular degeneration. Investigative ophthalmology & visual science. 2001 Apr 1;42(5):1051-6.
16. Bosch-Morell F, Villagrasa V, Ortega T, Acero N, Muñoz-Mingarro D, González-Rosende ME, Castillo E, Sanahuja MA, Soriano P, Martínez-Solís I. Medicinal plants and natural products as neuroprotective agents in age-related macular degeneration. Neural regeneration research. 2020 Dec;15(12):2207.
17. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy: the Beaver Dam Eye Study. Ophthalmology. 1992 Jun 1;99(6):933-43.
18. McCarty CA, Mukesh BN, Fu CL, Mitchell P, Wang JJ, Taylor HR. Risk factors for age-related maculopathy: the Visual Impairment Project. Archives of ophthalmology. 2001 Oct 1;119(10):1455-62.
19. Parekh N, Voland RP, Moeller SM, Blodi BA, Ritenbaugh C, Chappell RJ, et al CAREDS Research Study Group. Association between dietary fat intake and age-related macular degeneration in the Carotenoids in Age-Related Eye Disease Study (CAREDS): an ancillary study of the Women's Health Initiative. Archives of ophthalmology. 2009 Nov 9;127(11):1483-93.
20. Ancillotti C, Ciofi L, Pucci D, Sagona E, Giordani E, Biricolti S, et al . Polyphenolic profiles and antioxidant and antiradical activity of Italian berries from Vaccinium myrtillus L. and Vaccinium uliginosum L. subsp. gaultherioides (Bigelow) SB Young. Food chemistry. 2016 Aug 1;204:176-84.
21. Juadjur A, Mohn C, Schantz M, Baum M, Winterhalter P, Richling E. Fractionation of an anthocyanin-rich bilberry extract and in vitro antioxidative activity testing. Food chemistry. 2015 Jan 15;167:418-24.
22. González-Paramás AM, Brighenti V, Bertoni L, Marcelloni L, Ayuda-Durán B, González-Manzano S, Pellati F et al. Assessment of the in vivo antioxidant activity of an anthocyanin-rich bilberry extract using the Caenorhabditis elegans model. Antioxidants. 2020 Jun 10;9(6):509.
23. Ziberna L, Lunder M, Moze S, Vanzo A, Tramer F, Passamonti S,et al. Acute cardioprotective and cardiotoxic effects of bilberry anthocyanins in ischemia–reperfusion injury: Beyond concentration-dependent antioxidant activity. Cardiovascular Toxicology. 2010 Dec;10:283-94.
24. Šaponjac VT, Čanadanović-Brunet J, Ćetković G, Djilas S, Četojević-Simin D. Dried bilberry (Vaccinium myrtillus L.) extract fractions as antioxidants and cancer cell growth inhibitors. LWT-Food Science and Technology. 2015 May 1;61(2):615-21.
25. Bao L, Abe K, Tsang P, Xu JK, Yao XS, Liu HW, Kurihara H. Bilberry extract protect restraint stress-induced liver damage through attenuating mitochondrial dysfunction. Fitoterapia. 2010 Dec 1;81(8):1094-101.
26. Shi M, Loftus H, McAinch AJ, Su XQ. Blueberry as a source of bioactive compounds for the treatment of obesity, type 2 diabetes and chronic inflammation. Journal of Functional Foods. 2017 Mar 1;30:16-29.
27. Vaneková Z, Rollinger JM. Bilberries: Curative and miraculous–A review on bioactive constituents and clinical research. Frontiers in pharmacology. 2022 Jun 29;13:909914.
28. Riihinen K, Jaakola L, Kärenlampi S, Hohtola A. Organ-specific distribution of phenolic compounds in bilberry (Vaccinium myrtillus) and 'northblue'blueberry (Vaccinium corymbosum x V. angustifolium). Food chemistry. 2008 Sep 1;110(1):156-60.
29. Chan SW, Tomlinson B. Effects of bilberry supplementation on metabolic and cardiovascular disease risk. Molecules. 2020 Apr 3;25(7):1653.
30. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. Journal of nutritional science. 2016;5:e47.
31. Kähkönen MP, Heinämäki J, Ollilainen V, Heinonen M. Berry anthocyanins: isolation, identification and antioxidant activities. Journal of the Science of Food and Agriculture. 2003 Nov;83(14):1403-11.

32. Dashbaldan S, Becker R, Pączkowski C, Szakiel A. Various patterns of composition and accumulation of steroids and triterpenoids in cuticular waxes from screened Ericaceae and Caprifoliaceae berries during fruit development. Molecules. 2019 Oct 23;24(21):3826.
33. Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. Advances in pharmacological activities of terpenoids. Natural Product Communications. 2020 Mar;15(3):1934578X20903555.
34. Beatty S, Koh HH, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Survey of ophthalmology. 2000 Sep 1;45(2):115-34.
35. Hanus J, Zhang H, Wang Z, Liu Q, Zhou Q, Wang S. Induction of necrotic cell death by oxidative stress in retinal pigment epithelial cells. Cell death & disease. 2013 Dec;4(12):e965-.
36. Upadhyay M, Milliner C, Bell BA, Bonilha VL. Oxidative stress in the retina and retinal pigment epithelium (RPE): Role of aging, and DJ-1. Redox Biology. 2020 Oct 1;37:101623.
37. Datta S, Cano M, Ebrahimi K, Wang L, Handa JT. The impact of oxidative stress and inflammation on RPE degeneration in non-neovascular AMD. Progress in retinal and eye research. 2017 Sep 1;60:201-18.
38. Lau LI, Liu CJ, Wei YH. Increase of 8-hydroxy-2′-deoxyguanosine in aqueous humor of patients with exudative age-related macular degeneration. Investigative Ophthalmology & Visual Science. 2010 Nov 1;51(11):5486-90.
39. Shen JK, Dong A, Hackett SF, Bell WR, Green WR, Campochiaro PA. Oxidative damage in age-related macular degeneration. Histology and histopathology, vol. 22, nº12 (2007). 2007.
40. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, Rayborn ME, Salomon RG. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proceedings of the National Academy of Sciences. 2002 Nov 12;99(23):14682-7.
41. Lu L, Gu X, Hong L, Laird J, Jaffe K, Choi J, Crabb J, Salomon RG. Synthesis and structural characterization of carboxyethylpyrrole-modified proteins: mediators of age-related macular degeneration. Bioorganic & medicinal chemistry. 2009 Nov 1;17(21):7548-61.
42. Jeffrey BG, Weisinger HS, Neuringer M, Mitchell DC. The role of docosahexaenoic acid in retinal function. Lipids. 2001 Sep;36(9):859-71.
43. Gu X, Meer SG, Miyagi M, Rayborn ME, Hollyfield JG, Crabb JW, Salomon RG. Carboxyethylpyrrole protein adducts and autoantibodies, biomarkers for age-related macular degeneration. Journal of Biological Chemistry. 2003 Oct 24;278(43):42027-35.
44. Ethen CM, Reilly C, Feng X, Olsen TW, Ferrington DA. Age-related macular degeneration and retinal protein modification by 4-hydroxy-2-nonenal. Investigative ophthalmology & visual science. 2007 Aug 1;48(8):3469-79.
45. Terluk MR, Kapphahn RJ, Soukup LM, Gong H, Gallardo C, Montezuma SR, Ferrington DA. Investigating mitochondria as a target for treating age-related macular degeneration. Journal of Neuroscience. 2015 May 6;35(18):7304-11.
46. Blasiak J, Glowacki S, Kauppinen A, Kaarniranta K. Mitochondrial and nuclear DNA damage and repair in age-related macular degeneration. International journal of molecular sciences. 2013 Jan 31;14(2):2996-3010.
47. Kaarniranta K, Uusitalo H, Blasiak J, Felszeghy S, Kannan R, Kauppinen A, Salminen A, Sinha D, Ferrington D. Mechanisms of mitochondrial dysfunction and their impact on age-related macular degeneration. Progress in retinal and eye research. 2020 Nov 1;79:100858.
48. Ferrington DA, Ebeling MC, Kapphahn RJ, Terluk MR, Fisher CR, Polanco JR, Roehrich H, Leary MM, Geng Z, Dutton JR, Montezuma SR. Altered bioenergetics and enhanced resistance to oxidative stress in human retinal pigment epithelial cells from donors with age-related macular degeneration. Redox biology. 2017 Oct 1;13:255-65.
49. Iacovelli J, Rowe GC, Khadka A, Diaz-Aguilar D, Spencer C, Arany Z, Saint-Geniez M. PGC-1α induces human RPE oxidative metabolism and antioxidant capacity. Investigative ophthalmology & visual science. 2016 Mar 1;57(3):1038-51.
50. Decanini A, Nordgaard CL, Feng X, Ferrington DA, Olsen TW. Changes in select redox proteins of the retinal pigment epithelium in age-related macular degeneration. American journal of ophthalmology. 2007 Apr 1;143(4):607-15.
51. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Archives of ophthalmology. 2001 Oct 1;119(10):1417-36.
52. Age-Related Eye Disease Study 2 (AREDS2) Research Group. Lutein+ zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. Jama. 2013 May 15;309(19):2005-15.

53. Lin JB, Tsubota K, Apte RS. A glimpse at the aging eye. NPJ aging and mechanisms of disease. 2016 Mar 10;2(1):1-7.
54. Banerjee M, Chawla R, Kumar A. Antioxidant supplements in age-related macular degeneration: are they actually beneficial?. Therapeutic Advances in Ophthalmology. 2021 Aug;13:25158414211030418.
55. Tello, C. (2023). 7 Bilberry Benefits (incl. Eye Health) + Side Effects & Dosage. SelfDecode Supplements. https://supplements.selfdecode.com/blog/bilberry/
56. Burdulis D, Ivanauskas L, Dirsė V, Kazlauskas S, Ražukas A. Study of diversity of anthocyanin composition in bilberry (Vaccinium myrtillus L.) fruits. Medicina. 2007 Dec;43(12):971.
57. Bilberry extracts are not created equal: the role of non anthocyanin fraction. Discovering the "dark side of the force" in a preliminary study.
58. Gizzi C, Belcaro G, Gizzi G, Feragalli B, Dugall M, Luzzi R, Cornelli U. Bilberry extracts are not created equal: the role of non anthocyanin fraction. Discovering the “dark side of the force” in a preliminary study. European Review for Medical & Pharmacological Sciences. 2016 Jun 1;20(11).
59. Biedermann L, Mwinyi J, Scharl M, Frei P, Zeitz J, Kullak-Ublick GA, Vavricka SR, Fried M, Weber A, Humpf HU, Peschke S. Bilberry ingestion improves disease activity in mild to moderate ulcerative colitis—An open pilot study. Journal of Crohn's and Colitis. 2013 May 1;7(4):271-9.