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The Benefits of Antioxidant-Rich Fruits on Skin Health

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11.1 Introduction

The desire to look beautiful can be dated back to medieval times when women consumed arsenic and applied bat's blood in an attempt to improve their complexion. Attractive features have been repeatedly selected during evolution in the plant and animal kingdoms. Although Darwin was the first scholar to study human beauty standards from a biological standpoint in the 1800s, it was not until a century later that the cross-cultural beauty standards were validated [1]. Since then the cosmetic business has developed into a $280-billion global industry and has been projected to reach $313 billion by 2011 [2,3]. We now use sophisticated terms such as phytocosmetics, cosmeceuticals, dermaceuticals, and skinceuticals to describe beauty products whose purpose of usage are not at all unlike those utilized in ancient times. In modern society looking good is not only desirable but oftentimes necessary. The pursuit of physical beauty is no longer just for women; the men's grooming business has become the fastest growing segment in the beauty industry. Therefore, physical beauty is more than skin deep because beauty is intimately linked to healthy and radiant skin, which is an external reflection of one's overall inner health status.

Skin is the largest organ that is under constant assault by environmental oxidative stress including ultraviolet radiation (UVR), air pollutants, and chemical oxidants [4]. Thus, skin aging is an inevitable normal process. However, premature skin aging may occur due to factors such as external oxidative stress as well as smoking, imbalanced nutrition, excessive dieting, and mental stress [5]. In vitro and in vivo studies suggest that antioxidants regulate the biomarkers associated with premature aging by reducing oxidative stress including environmental stress such as ozone and cigarette smoking [5]. A double-blinded, placebo-controlled clinical study showed that oral and topical natural antioxidant treatments protect against the development of premature skin aging due to oxidative damage [6]. Therefore, natural fruits and vegetables rich in antioxidants may protect skin against premature aging. This chapter will focus on the beneficial effects of antioxidant-rich berries on skin health.

11.2 Oxidative Stress and Skin Aging

11.2.1 Free Radicals and Oxidative Stress

The free radical theory of aging, proposed by Dr. Harman, states that the reaction of active free radicals with cellular components initiates the changes observed in the aging process [7]. Reactive oxygen species (ROS) is one of the major types of free radicals. ROS is generated during normal aerobic metabolism [8]. Approximately 2–5% of the oxygen consumed by a cell is subsequently reduced to free radicals [9]. ROS production is normally neutralized by cellular antioxidant defense systems so as to maintain an equilibrium between the production and elimination of free radicals [10]. The endogenous antioxidant defense consists of enzymes such as superoxide dismutase, glutathione synthase, and catalase, as well as oxidant scavengers including glutathione (GSH) and uric acid. However, this defense mechanism is not entirely oxidant-proof. In fact, about 1% of the ROS elude the control of endogenous antioxidant defense systems daily, thus tipping the balance in favor of ROS accumulation [11]. This limitation is further exacerbated by the decline of endogenous antioxidant defense during aging. The end result, then, is an imbalance in cellular redox homeostasis, which leads to cellular oxidative damage in macromolecules such as DNA, lipids, and proteins [12]. The gradual accumulation of cellular oxidative damage results in oxidative stress. A myriad of investigations also suggest that oxidative stress may induce the expression of proinflammatory cytokines, which, in turn, may elevate the cellular levels of ROS [13]. This vicious cycle results in the progressive accrual of oxidative stress and inflammation during chronological skin aging [14]. Therefore, oxidative stress and inflammation appear to be a function of age. Although the aging process cannot be reversed as yet, it is possible to delay its onset by combating the accumulation of ROS and inflammation through exogenous sources of antioxidants, which can be obtained from fruits, vegetables, and dietary supplements [15–17].

11.2.2 Skin Aging and Skin Antioxidant Status

Normal skin aging is characterized by thinning of the epidermis, resulting in fine wrinkles, roughness, dry and thin appearance, hyperpigmentation, and seborrhoeic keratoses [5,18]. Although the underlying mechanisms for chronological skin aging remain incompletely understood, several causes including intrinsic factors such as genetic and epigenetic variations and diminished epidermal turnover, as well as extrinsic factors such as UVR, photo-oxidative stress, and ROS-induced collagen degradation and chronic inflammation have been proposed [14,18]. Accordingly, increased exposure to harsh environmental factors as well as reduced overall antioxidant status may accelerate the chronological skin aging process, leading to premature skin aging.

Because the skin is inevitably exposed to environmental oxidative stress, an adaptive enzymatic and nonenzymatic antioxidant defense network has been developed by the skin. The enzymatic defense includes superoxide dismutase, catalase, and glutathione synthase. Superoxide dismutase converts superoxide anion to hydrogen peroxide, which is further detoxified to water by catalase and glutathione synthase [19]. Nonenzymatic endogenous antioxidants in cutaneous cells consist of α-tocopherol (vitamin E), β-carotene (precursor of vitamin A), ascorbic acid (vitamin C), ubiquinon (coenzyme Q10), and glutathione. The lipophilic antioxidants α-tocopherol and β-carotene are found in the cell membranes and serve to protect against lipid peroxidation [20]. This defense system is crucial for normal cellular functions because lipid peroxides and their metabolites such as malonaldehyde and 4-hydroxy-2-nonenal may directly or indirectly induce detrimental consequences by evoking immune and inflammatory response, altering gene expression, and inducing apoptosis [20]. Because lipophilic α-tocopherol and hydrophilic ascorbate antioxidants cannot be synthesized de novo by humans, topical application or systemic administration of these and other antioxidants may be essential to replenish skin with antioxidants that are depleted by environmental assaults or by age-related increase in oxidative stress.

11.3 Cosmeceuticals, Antioxidants, and Skin Health

11.3.1 Cosmeceuticals

The term *cosmeceutical* was first coined by dermatologist Dr. Kligman to describe a product category between cosmetics and pharmaceuticals [21,22]. It is defined as a product that combines common cosmetic preparations with functional nutraceutical ingredients [23]. Although not yet recognized by the U.S. Food and Drug Administration, the term cosmeceutical has been used in the beauty industry for products containing natural ingredients and claiming health or physical improvement benefits beyond aesthetics [23,24]. Cosmeceuticals initially described cosmetic products that enhance beauty from the outside such as topical creams, lotions, and ointments. Today, the term *cosmeceutical* is extended to encompass a variety of drinks or dietary supplements known as skin nutraceuticals or nutricosmetics that provide additional health functions and benefits to enhance beauty from the inside [25].

The pursuit of physical beauty and overall well-being has boosted the demand for cosmeceuticals. In fact, cosmeceuticals have become the fastest growing sector of the natural personal care industry, commanding worldwide annual sales surpassing $14 billion [23]. The U.S. cosmeceutical category is a healthy $4.3 billion business, with a projected annual growth of approximately 8% [24]. Antioxidant ingredients are the largest segment of the cosmeceutical ingredient market. In 2000, antioxidants accounted for about 40% of the total cosmeceutical ingredient sales in the U.S. It is projected that antioxidants will continue to dominate the cosmeceutical market, with an annual growth rate of 7.3% [24].

11.3.2 Cosmeceutical Antioxidants

The use of antioxidants for skin care was well documented during the Renaissance by Paracelsus, who famously noted that "the dose makes the poison" and was often considered to be the father of toxicology [26]. He reportedly showed the beneficial effects of treating skin wrinkles with red wine vinegar, which, as we now know, contains grape polyphenolic antioxidants [21,27,28]. Currently antioxidants are widely incorporated into a variety of antiaging skin care systems [29]. The commonly used cosmeceutical antioxidant ingredients include vitamins A, B, C, and E; CoQ10 and its analogues; and plant polyphenols such as tannins and flavonoids.

11.3.3 Fruit Antioxidants and Skin Health

11.3.3.1 Selected Berry Antioxidants and Health Benefits

Anthocyanins are a subcategory of the flavonoid class that are water-soluble pigments present in all higher plants that give flowers and fruits their bright red, purple, or blue color. Anthocyanins have received a great deal of attention in recent years because of their antioxidant, anti-inflammatory, and antimutagenic effects [15]. There is a significant correlation between anthocyanin content and antioxidant capacity. Berry fruits are generally rich in anthocyanins. Consequently, berries are high in antioxidant capacity, which can be measured by the oxygen radical absorbance capacity (ORAC) assay [30–32].

Wild blueberries, one of the most popular *Vaccinium* species (spp.), have been shown to be among the foods that have the highest anthocyanin concentrations and ORAC values [33,34]. Numerous publications have indicated that blueberry anthocyanins exhibit a wide range of antiaging properties, such as reducing age-enhanced vulnerability to oxidative stress and inflammation, lowering the risk of developing age-related degenerative diseases, and improving overall brain function [15]. Other *Vaccinium* spp., such as bilberries and cranberries, are also enriched with anthocyanins and are capable of decreasing oxidative stress and inflammation [33,35–38].

The antioxidant effect of elderberry (*Sambucus* spp.) anthocyanins on endothelial cells was evaluated by challenging the cells with oxidative stressors such as hydrogen peroxide, azobis(2-amidinopropane) dihydrochloride, and iron sulfate. Endothelial cells preloaded with elderberry extract were significantly protected against insults of the oxidative stressors [39]. Elderberry extract at a low concentration was found to give a considerable amount of antioxidant protection against both copper-induced low-density lipoprotein oxidation and peroxyl radical attack [40]. Therefore, elderberry extract could contribute significantly to the antioxidant capacity of plasma [40].

The Rosaceae family berries, such as strawberries and raspberries, are also high in antioxidant capacity. Ellagic acid is the predominant phenolic antioxidant found in strawberries (*Fragaria* spp.). It has been estimated that ellagic acid accounts for approximately 51% of the total phenolic compounds in strawberries [41]. A comparative study was conducted to determine the total antioxidant capacity of 12 fruits, and strawberries were found to rank the highest in ORAC value among the other nonberry fruits [42]. A clinical study showed that consumption of strawberries increased the serum antioxidant capacity in elderly women [43]. Individual constituents of strawberries appear to afford protective potential against cancer and cardiovascular disease while enhancing mental health and boosting the immune system [44]. Raspberry (*Rubus* spp.) seeds, the by-product of juice manufacturing, contain a significant amount of phenolic antioxidants and omega-3 unsaturated fatty acids [45]. Omega-3 fatty acids are themselves high in antioxidant capacity [46]. The health benefits of omega-3 fatty acids include antiatherosclerosis, cardioprotection, anticarcinogenesis, antioxidative defense, anti-inflammation, autoimmune disease prevention, and mental health enhancement [47–51].

11.3.3.2 Evidence-Based Approach to Evaluating the Benefits of a Novel Berry Formulation

Because individual berries confer different health benefits, an innovative formulation combining different berry extracts at specific proportions was systematically researched and developed for its health-promoting effects. The comparative antioxidant efficacy was determined for extracts from the fruits of wild blueberries, wild bilberries, cranberries, elderberries, and strawberries, as well as extracts from seeds of raspberries. A total of 20 different combinations comprising varying amounts of each of the extracts from these six berries were evaluated by the ORAC assay [52]. The blend with the highest ORAC value was selected for further analysis. The cytotoxicity of this formulation was assessed by the lactate dehydrogenase assay using human epidermal keratinocyte (HaCaT) cells to ensure the formulation was not toxic to the cells. Keratinocytes are the major cell type of the epidermis, comprising up to 90% of the skin cells. The lactate dehydrogenase assay indicated that the formulation was not cytotoxic to epidermal cell viability as compared to control and each of the six berry extracts [52].

The safety and efficacy properties of this formulation, known as OptiBerry, were further investigated using standard toxicology and whole-body antioxidant status procedures [53]. The results indicated that acute oral LD_{50} of OptiBerry was greater than 5 g/kg body weight whereas acute dermal LD_{50} was greater than 2 g/kg body weight in rats [53]. The antioxidant potential of OptiBerry was evaluated in vitamin E–deficient rats by determining glutathione (GSH) oxidation after exposure to clinically relevant hyperbaric oxygen (HBO) at 2 atmospheric pressure (atm) for 2 hours. Rats fed an OptiBerry-supplemented diet for 8 weeks were significantly protected against HBO-induced GSH oxidation in the lung and liver as compared to placebo-fed rats. The antioxidant property of OptiBerry was further confirmed by a state-of-the-art electron paramagnetic resonance (EPR) technology, which is an imaging system used to examine the whole-body redox status. Vitamin E–deficient mice were subjected to HBO treatment at 2 atm for 2 hours. Mice were then anaesthetized, injected with carbamoyl-PROXYL (nitrosyl radical) solution, and placed into quartz tubes. Antioxidants reduce nitrosyl radicals to hydroxamine, thereby accelerating the EPR signal decay of nitrosyl radicals. To determine the reduction of nitrosyl radicals in vivo, EPR spectra were immediately recorded on the body of the test compound–injected mice at 4-min intervals and a total of 16 projections were taken for each time point. The projections for each interval were deconvoluted to reconstruct images of two-dimension redox status [53]. Mice fed an OptiBerry-supplemented diet for 2 weeks exhibited significant protection against HBO-induced oxidative stress as revealed by the rapid decay of EPR signal intensity [53]. Therefore, the data suggest that OptiBerry is safe and may provide whole-body antioxidant protection.

Angiogenesis is a process involving the generation of new blood vessels from pre-existing vessels in areas of low blood supply [54]. It is a hallmark in tumor growth and cancer metastases. The skin vasculature remains quiescent under normal conditions. However, under external insults such as UVR and heat, skin inflammation, and cancer, as well as during wound healing and hair growth, the skin cells are capable of initiating rapid angiogenesis. The epidermis-derived vascular endothelial growth factor (VEGF) is a potent angiogenic factor. Its expression can be altered by a variety of physiological and pathological conditions of the skin [54]. It has been shown that VEGF expression was stimulated by UVR in epidermal keratinocyte cell lines and in human skin [55,56]. Heat treatment at 43°C for 90 minutes in human volunteers has also been shown to induce an angiogenic switch through upregulation of VEGF protein expression with concomitant increase in vascularization [57]. Therefore, skin angiogenesis is associated with an array of acute external stimuli, which may lead to vascular hyperpermeability, resulting in cutaneous inflammation and progressive loss of skin vessels in aged skin [54].

The inhibitory effect of OptiBerry on angiogenesis was revealed by the level of VEGF expression in HaCaT keratinocytes. In this experiment, HaCaT cells were pretreated with or without Optiberry for 12 hours, followed by treatment with H_2O_2 and tumor necrosis factor-alpha (TNF-α, an inflammatory cytokine). OptiBerry pretreatment was found to significantly inhibit the ROS- and inflammation-induced VEGF protein expression [58,59]. In addition, the formation of endothelial tubes, an indication of angiogenesis, was investigated using Matrigel assay. Human microvascular endothelial cells cultured on Matrigel exhibit complex morphological behavior by reconstructing intricate spider weblike networks resembling the vasculature systems. Treatment with OptiBerry inhibited the construction of this network by reducing the formation of endothelial tubes, corroborating the antiangiogenic property of OptiBerry [58,59]. Unexpectedly, grape seed proanthocyanidin did not elicit the antiangiogenic activity observed for OptiBerry, suggesting that antioxidant capacity alone may not be sufficient to account for the antiangiogenic effect. Therefore, OptiBerry may exert its antiangiogenic property through other means such as transcriptional activation/inhibition in conjunction with its antioxidant potential [58]. This theory was subsequently confirmed in the following experiments.

Hemangiomas are the most common form of infant tumors, affecting 10–12% of normal newborns. Approximately 5% of hemangiomas cause serious tissue damage, and 1–2% of all hemangiomas are life-threatening. Hemangiomas are localized tumors of blood vessels characterized by a rapid proliferation of capillaries during the newborns' first year [60]. The underlying causes for the endothelial cell growth in hemangiomas have not been determined, although proliferating hemangiomas have been shown to be highly angiogenesis-dependent [61,62]. Also, hemangiomas are infiltrated by macrophages, which can initiate angiogenesis. The chemokine monocyte chemoattractant protein-1 (MCP-1) is responsible for recruiting macrophages at the lesion sites, thus facilitating the growth of hemangiomas and vascular malformations [63,64]. Both VEGF and MCP-1 were found to be highly stimulated in proliferative hemangiomas [63,65]. In fact, transgenic rabbits with increased hepatic expression of the human VEGF transgene under the control of the human alpha-antitrypsin promoter were shown to develop liver hemangiomas resembling the Kasabach-Merritt syndrome [66]. In this regard, hemangioma represents a powerful model for the investigation of angiogenesis and for the identification of anti-angiogenic molecules, which arrest endothelial cell growth at a quiescent state under physiological conditions.

Endothelioma cells (EOMA) are derived from the spontaneously arising hemangio-endothelioma in the 129/J murine strain [67]. EOMA cells treated with OptiBerry for 12 hours were significantly protected against TNF-α-induced MCP-1 upregulation [67]. In order to evaluate the molecular mechanisms underlying the observed inhibitory effects of OptiBerry on the expression of MCP-1, EOMA cells were transfected with an MCP-1-controlled luciferase reporter construct. These transfected cells exhibited high basal luciferase expression, indicating an elevated MCP-1 transcription [68]. Pretreatment of the transfected cells with OptiBerry significantly lowered the basal MCP-1-luciferase reporter expression, suggesting that OptiBerry inhibited the basal transcription of MCP-1 in EOMA cells. Because MCP-1 is under the control of transcription factor nuclear factor kappa B (NF-κB), examination of whether OptiBerry affects the activation of NF-κB should provide insight into the upstream events in the cascade leading to the regulation of MCP-1. Consequently, EOMA cells were transfected with NF-κB luciferase construct, pretreated with or without OptiBerry for 24 hours, and then challenged with TNF-α for 6 hours to induce inflammation. Transcriptional activation of NF-κB was assayed by measuring the level of NF-κB–controlled luciferase activity. Transfected cells not pretreated with OptiBerry showed a marked increase in the level of NF-κB–luciferase activity; however, this increase was significantly attenuated by pretreatment of OptiBerry [68]. The findings demonstrated for the first time that berry constituents regulated NF-κB activity in EOMA cells.

Mice injected with EOMA cells develop clinically identifiable hemangiomas resembling the Kasabach-Meritt syndrome within 3–4 weeks, whereas tumor formation only takes days. Therefore, mice injected with EOMA cells provide an in vivo model for the study of angiogenic events. To investigate the in vivo effect of OptiBerry on angiogenesis, mice were injected with EOMA cells with or without OptiBerry pretreatment. Mice (129P3/J) were sacrificed one week post-injection to obtain tissues for histological analyses. Although the OptiBerry treated group tested positive for the presence of hemangiomas, the average mass of such tumor growth was below 50% of those in the untreated control group. Immunohistological analysis revealed a significant reduction in the infiltration of macrophages in hemangioma of treated mice as compared to that of the controls [68]. It appears that the beneficial action of OptiBerry on proliferating hemangiomas may, in part, result from reduced MCP-1 expression and reduced influx of angiogenic macrophages. OptiBerry may therefore represent a potent agent against angiogenesis-induced skin aging.

11.4 Conclusion

The beauty industry has come a long way. The current trend for skin care products is that they not only conceal superficial blemishes but also treat the causes from within. At present the consensus on food is two-fold: it provides essential nutrients to sustain life itself and supplies bioactive agents to promote health and prevent diseases. A large body of evidence has demonstrated the correlation between consumption of fresh fruits and vegetables with delaying and/or preventing chronic degenerative diseases. Fresh fruits and vegetables are enriched with a variety of diverse nutrients, such as vitamins, antioxidants, trace minerals and micronutrients, phytosterols, phytoenzymes, dietary fiber, and potent chemoprotectants [69]. Epidemiological studies have consistently revealed the beneficial effects of nutritional factors on the prevention of chronic diseases and the modulation of human skin conditions [70–72]. In recent years, functional foods and nutraceuticals have gained noticeable popularity. Both oral and topical supplements have become some of the most widely used alternative therapies. Antioxidants make up a majority of the skin health ingredients in the cosmeceutical market because oxidative stress is generally accepted as a major contributing factor to skin aging. Consuming natural antioxidants provides a plethora of health benefits, including lowering age-related oxidative stress and inflammation [15,73].

Anthocyanins are the common components of fruits and vegetables, particularly in berries, which provide the bright red, blue, and purple hues to the plants. Anthocyanins are naturally occurring antioxidants. There is a direct correlation between anthocyanin content and antioxidant capacity in berry fruits [36]. In vitro and in vivo studies have shown that berry anthocyanins possess potent antioxidant activity and many potential health benefits, including cardiovascular protection, anticarcinogenic potential, antidiabetic properties, brain function enhancement, ocular and vision health, urinary tract health, and skin health [74]. Based on these health benefits, a formulation of a synergistic blend (OptiBerry) containing wild blueberry, bilberry, cranberry, elderberry, strawberry, and raspberry seed extracts was developed. Systematic and comprehensive studies have shown that OptiBerry is safer and more potent than individual and all other combinations of the berry extracts tested [58]. Furthermore, OptiBerry has been found to be superior in bioavailability and antioxidative properties, providing whole-body antioxidant protection [53,59].

It has been shown that the skin is capable of exiting the quiescent phase of vasculature to rapidly initiate angiogenesis during external insults such as UVR and thermal stimulus [54]. This surge of acute vessel development incurs profound consequences to the general health of the aging skin because these vessels may be leaky and less mature, which may lead to vascular hyperpermeability and vessel leakage, resulting in skin inflammation and further degradation of the extracellular matrix [54]. Both VEGF and MCP-1 are markedly activated during angiogenesis. Several in vitro and in vivo studies have shown that OptiBerry significantly reduced the expression of VEGF and MCP-1 in highly proliferative hemangioma, an angiogenesis-dependent disease. The inhibitory effect of OptiBerry on angiogenesis was attributable to the modulation of VEGF and MCP-1 through the activation of upstream transcription factor NF-κB [58,59,68]. This finding is of great importance because it demonstrates that, in addition to its antioxidant activities, OptiBerry anthocyanin constituents may serve as signal molecules to modulate gene expression. This observation is consistent with the findings that flavonoids and their metabolites may modulate various protein kinase signaling pathways [75].

Is beauty only skin deep? The answer is apparent. Although visible signs of aging are readily reflected on the surface of the skin as the consequence of time and environmental insults, the fundamental causes may very well be more than skin deep. To achieve overall well-being, antiaging treatment should start from the inside to reduce oxidative stress and inflammation, curtail degenerative diseases, and attenuate age-related decline in cognitive and motor functions, which will then translate to the outside as a vibrant and healthy appearance. In this regard, consuming foods rich in antioxidants and other phytonutrients may help to promote beauty from within.

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