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Original Article

Anti-wrinkle activity of the ethanol extract of *Aronia melanocarpa* for Development of the cosmeceutical ingredients

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Abstract

Despite the abundance of natural biological resources in *Aronia melanocarpa*, there is still insufficient research specifically exploring the potential for developing cosmeceutical compositions utilizing this plant. To develop the ingredient with the cosmeceutical function, the anti-wrinkle effect of the ethanol extract of *A. melanocarpa* was investigated. The ethanol extract was prepared from the dried *A. melanocarpa*. DPPH radical scavenging activity was significantly increased by the ethanol extract of *A. melanocarpa*. Cell viability on CCD986Sk human fibroblast was not affected by the ethanol extract of *A. melanocarpa*. Moreover, the ethanol extract of *A. melanocarpa* demonstrated inhibition of both collagenase and elastase enzymes. Analysis through ELISA and western blotting revealed an elevation in collagen expression levels in CCD986Sk human fibroblasts treated with ethanol extract. Additionally, the extract induced migration and invasion in HaCaT human keratinocytes, potentially associated with the activation of tight junctions. These results offer insights for the development of innovative cosmeceutical formulations utilizing *A. melanocarpa* extract.

Keywords: *Aronia melanocarpa*, Anti-wrinkle, Collagen, Collagenase, Elastase

1. Introduction

Aronia melanocarpa, commonly known as black chokeberry, has been recognized for its potential health benefits due to various bioactive compounds [1]. *A. melanocarpa* is known for its high antioxidant content, including anthocyanins, flavonoids, and polyphenols. Antioxidants in *A. melanocarpa* may help improve cardiovascular health by reducing oxidative stress, supporting blood vessel function, and potentially lowering blood pressure [2]. *A. melanocarpa* extracts have been studied for their potential to modulate the immune system [3]. In addition, some research suggests that *A. melanocarpa* may possess anti-cancer properties [4] and help regulate blood sugar levels and improve insulin sensitivity [5]. Even though *A. melanocarpa* shows promise in various health aspects, there is not enough research on the anti-winkle activity of *A. melanocarpa* to fully understand its mechanisms of action and to establish specific recommendations for its use as cosmeceutical ingredients.

Cosmeceuticals are cosmetic products that contain bioactive ingredients with potential therapeutic benefits for the skin [6]. Cosmeceuticals often contain biologically active compounds such as antioxidants, peptides, vitamins, and growth factors designed to provide benefits beyond basic skincare. Many cosmeceuticals focus on anti-aging effects, aiming to reduce the appearance of fine lines, wrinkles, and other signs of skin aging [7]. Some cosmeceuticals include ingredients that promote the synthesis of collagen and elastin, crucial proteins for skin elasticity and firmness [8, 9]. Cosmeceuticals may incorporate ingredients like vitamin C or alpha-arbutin to address hyperpigmentation and promote a more even skin tone [10, 11]. Sunscreen-containing cosmeceuticals protect against harmful UV rays, helping prevent sun damage and premature aging [12].

Wrinkles in the skin are a natural part of the aging process and can also be influenced by various external factors [13]. The formation of wrinkles involves a combination of intrinsic and extrinsic factors [14]. Collagenase is an enzyme that plays a significant role in the remodeling and turnover of collagen, a crucial protein in the skin's structure. It specifically targets collagen by breaking down its peptide bonds, leading to the degradation of collagen fibers. In the skin, collagenase contributes to processes such as wound healing and tissue repair by clearing damaged or old collagen, allowing for the synthesis of new collagen. While regulated collagen degradation is essential for maintaining skin health, imbalances in collagenase activity can contribute to skin disorders and aging. Research into collagenase regulation and its implications in skin

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biology is ongoing for potential therapeutic applications [8, 15]. Elastase is an enzyme that plays a role in the maintenance of skin elasticity. It primarily targets elastin, a protein responsible for the resilience and flexibility of the skin. Elastase breaks down elastin fibers by cleaving their peptide bonds, contributing to the natural turnover and remodeling of the extracellular matrix. While controlled elastase activity is essential for skin function, excessive or unregulated elastase activity can lead to the degradation of elastin, contributing to skin aging and loss of elasticity. Understanding and regulating elastase activity is a focus of skincare research, to develop strategies to preserve skin elasticity and prevent premature aging [16]. Thus, Scientists have endeavored to create innovative methods to hinder the activity of collagenase and elastase enzymes to enhance skin tensile firmness, potentially paving the way for the formulation of cosmeceuticals with anti-wrinkle [17].

In this study, we assessed the potential of ethanol extracts from *A. melanocarpa* (AME) as a cosmeceutical ingredient. AME demonstrates anti-wrinkle effects by inhibiting collagenase and elastase while promoting the upregulation of protein markers associated with tight junctions in skin cells. Consequently, our ongoing research suggests that AME could serve as a promising agent for treating skin disorders by supporting skin tissue maintenance.

2. Materials and Methods

2.1. Preparation of the ethanol extract of *A. melanocarpa (AME)*

Dried *A. melanocarpa* was ground and sifted through a 30-mesh sieve (600 μm particle size). Then, the powder was extracted with 70% ethanol (Sigma Aldrich, St. Louis, MO, USA) and concentrated by rotary evaporation. The extract was dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich, 100 mg/ml) and kept in -20°C.

2.2. Chemicals

Ascorbic acid, retinoic acid, glycerol, glycine, sodium chloride, Trizma base, and Tween 20 were from Sigma Aldrich.

2.3. Cell lines

CCD986Sk and HaCaT from ATCC (Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma Aldrich) and 1% streptomycin/penicillin (Sigma Aldrich) in a humidified CO2 incubator at 37°C.

2.4. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

180 μl of DPPH solution (Sigma Aldrich) in methanol were mixed with 20 μl of AME (100, 50, and 25 μg/ml) or ascorbic acid (A.A., 1 μl) in a 96-well plate. The plate was stored in the dark for 15 min and the absorbance was measured at 517 nm using the microplate reader (Molecular Devices, Mountain View, CA, USA).

2.5. WST-1 assay

CCD986Sk (1×10^3 cells) was seeded into each well of a 96-well plate and incubated for 18 h. Then, cells were treated with AME (100, 50, and 25 μg/ml) or retinoic acid (R.A., 10 μM) for 48 h. 20 μl of WST-1 solution (Daeillab Service, Seoul, Korea) was added to each well. After 4 h, the absorbance was measured at 460 nm using a microplate reader (Molecular Devices).

2.6. Collagenase assay

150 μl of collagenase (0.2 mg/ml, Abcam, Cambridge, UK) was mixed with 100 μl of AME (100, 50, and 25 μg/ml) or (1, 10)-phenanthrolin (Phe., collagenase inhibitor, 10 μM, Abcam) in a 1.5 ml tube followed by addition of 250 μl of 4-phenylazobezyloxylcarbonyl Pro-Leu-Gly-Pro-Arg (collagenase substrate, 0.3 mg/ml, Abcam). After incubation at 25°C for 20 min, the reaction was stopped by addition of 6% citric acid (Sigma Aldrich). The reaction mixture was extracted by ethyl acetate (Sigma Aldrich). The absorbance was measured at 320 nm using the microplate reader (Molecular Devices).

2.7. Elastase assay

25 μl of elastase solution (0.2 mg/ml, Abcam) was mixed with 100 μl of AME (100, 50, and 25 μg/ml) or elastatinal (Ela., elastase inhibitor, 10 μM, Abcam) in a 96-well plate followed by addition of 25 μl of elastase substrate. After incubation at 25°C for 30 min, the absorbance was measured at 410 nm using the microplate reader (Molecular Devices).

2.8. ELISA

CCD986Sk (1×10^5 cells/well) were seeded to a 6-well plate and incubated for 18 h. Then, the culture medium was changed to serum-free medium containing AME (100, 50, and 25 μg/ml) and incubated for 48 h. The amount of procollagen type I C-peptide in culture medium was measured with a procollagen type I C-peptide ELISA kit (Takara, Otsu, Japan) according to manufacturer's manual.

2.9. Western blotting analysis

CCD986Sk (1×10^5 cells/well) were seeded to a 6-well plate and incubated for 18 h. Then, the culture medium was changed to serum-free medium containing AME (100 and 50 μg/ml) and incubated for 48 h. The total proteins in culture medium were concentrated by Centricon (100 kDa, Sigma Aldrich). HaCaT (1×10^5 cells/well) were seeded to a 6-well plate and incubated for 18 h. Then, cells were treated with AME (100 μg/ml) for 48 h. The total proteins separated by electrophoresis on 10% SDS polyacrylamide gel, transferred to a PVDF membrane (Sigma Aldrich) and analyzed by the anti-collagen Type I (1:500, Sigma Aldrich), anti-ZO-1 (1:1000, Sigma Aldrich), anti-Occludin1 (1:1000, Sigma Aldrich), and anti-Claudin-1 (1:1000, Sigma Aldrich) antibodies. After harvesting cells, Membrane was incubated using and horse radish peroxidase-conjugated secondary antibody (Sigma Aldrich). Chemiluminescence was detected using enhanced chemiluminescent substrate (Sigma Aldrich).

2. 10. Wound healing assay

HaCaT (1×10^5 cells/well) were seeded in 6-well plates and incubated with serum-free medium for 18 h. Then, an artificial wound was scratched into the cell using a P20 pipette tip. Then media was changed with DMEM with 1% FBS containing AME (100 μg/ml) or R.A. (10 μM). Images were taken by microscopy (Leica, GmbH, Germany) at 0 and 48 h.

2.11. Invasion assay

HaCaT (1×10^5) were seeded in the upper chamber of

invasion assay plate (pore size: 8 µm, Corning Life Sciences, Lowell, MA, USA) with serum-free medium for 18 h. After treating cells with AME (100 µg/ml) or R.A. (10 µM), the invaded cells were fixed with 4% paraformaldehyde solution (Sigma Aldrich) and stained with 1% crystal violet solution (Sigma Aldrich). Images were taken by microscopy (Leica) at 48 h.

2.12. Statistical analysis

All experiments were performed in triplicate. The data are expressed as the mean ± standard deviation (SD). Significant differences between controls and treated cells were analyzed by student's t-test.

3. Results

3.1. Effect of AME on DPPH radical scavenging activity and CCD986Sk viability.

Antioxidant effect of natural plant extracts is well-known standard to determine whether the extract can be used as a component of the cosmeceutical formulation or not. As shown in Figure 1.A, it was demonstrated that AME showed DPPH radical scavenging activity in dose-dependent manner. Especially, 100 and 50 µg/ml of AME have statistically significant (p-value < 0.05) DPPH radical scavenging activity compared to the control. 100 µg/ml of AME has a slightly lower DPPH radical scavenging activity than 1 µM of ascorbic acid that was used for the positive control. To investigate the cell viability of AME on CCD986Sk human fibroblast cells known to express the proper collagen expression in skin [18, 19], CCD986Sk cells were treated with AME and the viability was measured using the WST-1 assay. As shown in Figure 1. B, AME treatment did not change the cell growth rate of CCD986Sk up to 100 µg/ml. Thus, these results suggest that AME has the potential to provide more collagen protein around skin fibroblasts without any toxicity.

3.2. Effect of AME on collagenase and elastase activity.

In order to develop the anti-wrinkle ingredients for cosmeceuticals, we investigated the effects of AME on collagenase activity. As shown in Figure 2. A, AME decreased collagenase activity with dose dose-dependent way. The collagenase activity was decreased up to approximately 18% compared to the control at 100 µg/ml of AME, which was a slightly lower value than that of the positive control (10 µM of 1,10-Phenanthroline). Then, we tried to examine whether AME inhibits elastase activity or not. As shown in Figure 2.B, 100 µg/ml of AME decreased elastase activity with an approximate 60% compared to the control. 10 µM of elastatinal as a positive control showed 50% inhibition rate. Thus, these results indicated that AME had the potential to inhibit both collagenase and elastase which was known to be involved in the regulation of winkle formation and tissue elasticity.

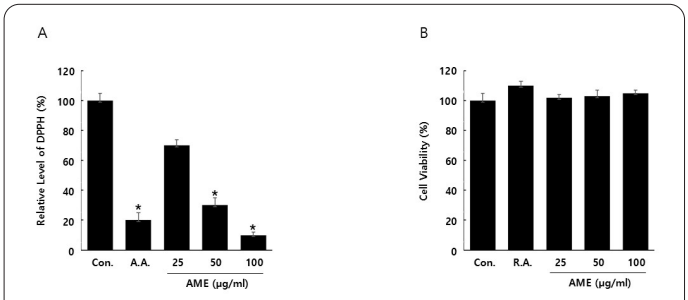

Fig. 1. Anti-oxidant activity and investigation of CCD986Sk cell viability of the ethanol extract of *A. melanocarpa* (AME). (A) DPPH radical scavenging ability of AME. The concentration of DPPH radical was determined using 100, 50, and 25 µg/ml of AME with a DPPH solution. Ascorbic acid (A.A., 1 µM) was used as a positive control. (B) Effects of AME on the viability of CCD986Sk cells. Cells were treated with 100, 50, and 25 µg/ml of AME for 48 h and viability was determined by WST-1 assay. Retinoic acid (R.A., 10 µM) was used as a positive control. Statistical differences between control vs. group * < 0.05.

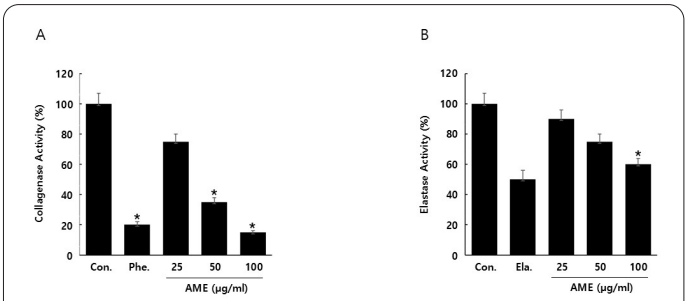

Fig. 2. Anti-collagenase and elastase activity of AME. (A) Collagenase inhibitory activity of AME. Collagenase was reacted with 100, 50, and 25 µg/ml of AME. 1,10-phenanthroline (Phe., 10 µM) was used as a positive control. (B) Elastase inhibitory activity of AME. Elastase was reacted with 100, 50, and 25 µg/ml of AME. Elastatinal (Ela., 10 µM) was used as a positive control. Statistical differences between control vs. group * < 0.05.

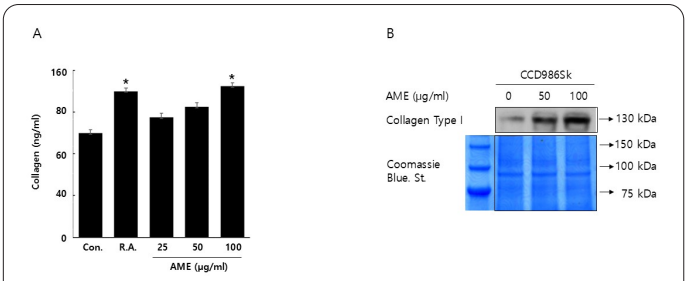

Fig. 3. Collagen synthesis activity of AME. (A) Effect of AME on collagen synthesis in CCD986Sk cells. Cells were treated with 100, 50, and 25 µg/ml of AME for 48 h and collagen amount was determined by ELISA. Retinoic acid (R.A., 10 µM) was used as a positive control. Statistical differences between control vs. group * < 0.05. (B) Western blotting analysis of collagen expression level. Cells were treated with 100 and 50 µg/ml of AME for 48 h and collagen Type I in the cell culture media was analyzed. Coomassie blue staining analysis was used for the loading control.

3.3. Effect of AME on collagen synthesis in CCD986Sk.

After confirming the anti-collagenase activity of AME, we tried to examine the amount of collagen in CCD986Sk cell culture media treated by AME. The level of Type I collagen was measured by procollagen Type I C-peptide ELISA assay kit since the amount of procollagen secreted from CCD986Sk cell reflects the amount of collagen. As shown in Figure 3.A, AME increased the expression of Type I collagen in CCD986Sk cell culture media in a dose-dependent way. 100 µg/ml of AME treatment induced the secretion of about 145 ng/ml of Type I collagen (p-value < 0.05), which was slightly higher than that (140 ng.ml) of 10 µM of retinoic acid treatment as the positive control. In addition, the expression of Type I collagen was confirmed by Western blotting analysis (Figure 3. B). Type I collagen

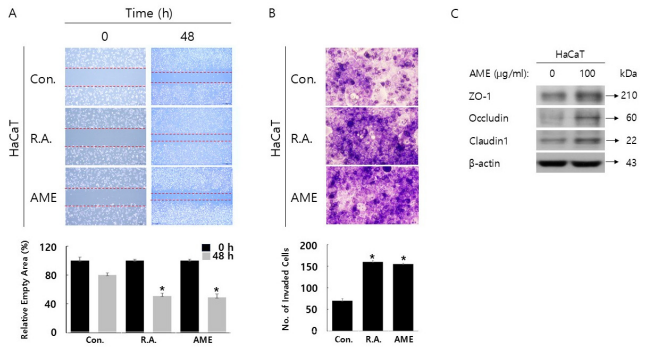

Fig. 4. Investigation of HaCaT cell mobility of AME. (A) Effect of AME on migration of HaCaT. Cells were treated with 100 μg/ml of AME for 48 h and migration of cells was observed by microscopy. (B) Effect of AME on invasiveness of HaCaT. Cells were treated with 100 μg/ml of AME for 48 h and invasiveness of cells was observed by microscopy. Retinoic acid (R.A., 10 μM) was used as a positive control. Migration or invasiveness was presented as bar graph. Statistical differences between control vs. group * < 0.05. (C) (A) Effect of AME on tight junction related protein expression in HaCaT. Cells were treated with 100 μg/ml of AME for 48 h and expression of ZO-1, Occludin, and Claudin1 was analyzed by each antibody. β-actin was used as a loading control.

was collected from the cell culture media under the same condition with ELISA analysis and analyzed by anti-Type I collagen antibody. In fact, compared to the control, the expression of Type I collagen was increased by AME treatment (50 and 100 μg/ml) in CCD986Sk cells.

3.4. Effect of AME on cell mobility in HaCaT.

To investigate the effect on keratinocyte mobility by AME, wound healing assay was performed. As shown in Figure 4. A, assay results showed that the gap of wound was more narrowed by AME than the control in HaCaT cells. In addition, invasion assay results indicated that the number of membrane-traversed cells was increased by AME in HaCatT cells (Figure 4. B). 100 μg/ml of AME treatment induced a similar activity of migration and invasion of HaCaT cells with 10 μM of retinoic acid treatment as the positive control. Then, we analyzed the expression level of Claudin1, Occludin, and ZO-1 which were tight junction (TJ) proteins responsible for adhesive closure functions in skin keratinocytes. As shown in Figure 4. C, the expression of Claudin1, Occludin, and ZO-1 was upregulated by AME in HaCaT cells. Thus, these results suggest that the enhanced cell mobility by AME is associated with TJ function in skin HaCaT human keratinocytes.

4. Discussion

Extracts from traditional herbal plants are gaining attention for cosmetic uses, particularly in applications such as anti-wrinkle and skin whitening. *A. melanocarpa*, a traditional herbal remedy in Asia for treating diverse diseases, has potential functional ingredients for cosmeceuticals. However, the novel activity of AME is not fully comprehended, and the molecular mechanisms underlying its effects are yet to be elucidated. Our research aims to explore the anti-wrinkle properties of AME as part of the effort to develop new cosmeceutical ingredients, contributing to advancements in cosmetic substances derived from this plant. There have been several research on the biological activity of AME. Application of a topical extract from A. melanocarpa, abundant in chlorogenic acid and rutin, mitigates UVB-induced skin damage in mice by alleviating disruptions in collagen [20]. AME enhances the production of type I collagen while reducing the expression of MMP1 and MMP3 in HaCaT cells [21]. In addition, a recent study showed that consuming AME in cases of cadmium intoxication could be effective in preventing disruptions in collagen homeostasis induced by this xenobiotic in the liver [22]. Thus, AME has been reported to stimulate collagen synthesis in the skin and liver. Similar to the previous studies, ELISA analysis indicated that AME elevated the expression of Type I collagen in a dose-dependent manner (Figure 3. A). This was verified through Western blotting analysis of Type I collagen obtained from the cell culture media (Figure 3. B).

Collagen represents a type of extracellular matrix protein present in connective tissues, constituting approximately 30% of the total proteins in mammals [23, 24]. Human tissues contain different types of collagen, with Type I collagen recognized for its pivotal role in the process of skin recovery. [25]. Collagen originates from precursor procollagen, which incorporates additional peptide sequences at both the N and C-termini. [25]. Following the removal of the extra peptide sequence from procollagen by procollagen peptidase, collagens are produced as components of extracellular matrix proteins and integrated into the mesh network of extracellular fibrils [26]. External factors such as UV exposure and aging markedly increase the expression of matrix metalloproteinases, also known as collagenases responsible for degradation of collagen [27]. For instance, subjecting skin fibroblasts to excessive UV irradiation stress can trigger the activation of Erk kinase, subsequently activating the c-Jun transcriptional factor. Consequently, the elevated expression of collagenase occurs, contributing to the degradation of collagen and the initiation of wrinkle formation in the skin dermis [19]. Elastin is a constituent of the extracellular matrix found in the connective tissue surrounding the skin dermis, and it can undergo degradation by elastase, a type of proteinase enzyme [28]. Hence, controlling collagenase and elastase activity could safeguard the elasticity of skin connective tissue and potentially serve as a therapeutic approach for various diseases. As shown in Figure 2. A and B, 100 μg/ml of AME reduced the activity of collagenase by about 18% which was similar to the positive control (10 μM of 1,10-Phenanthroline). Also, 100 μg/ml of AME demonstrated an approximate 60% decrease in elastase activity. The positive control, elastatinal at 10 μM, exhibited a 50% inhibition rate.

The adhesive closure functions of TJ are essential for ensuring the nterconnectedness of skin cells and regulating the movement or permeation of substances between these neighboring cells [29]. An actual consequence of the improved TJ function in the skin is the heightened hydration in the surrounding area, which is likely associated with the regulation of wrinkle formation. Tight junctions involve constitutive proteins integrated into cell membranes and cytosolic proteins, including Claudins, Occludins, JAMs, and ZO-1. In the context of skin TJ, among numerous Claudin isomers, Claudin1 is recognized as the most crucial protein for TJ regulation. Consequently, we conducted an analysis of the expression levels of Claudin1, Occludin, and ZO-1 in HaCaT human keratinocytes treated with AME using Western blotting. As depicted in

Figure 4. C, AME upregulated Claudin1, Occludin, and ZO-1 in HaCaT cells. This finding was corroborated by a wound-healing migration assay, as the enhanced TJ function can influence cell mobility and migration. [30] (Figure 4. A and B).

5. Conclusion

In this research, we demonstrated that AME had no impact on the growth of CCD986Sk human fibroblasts, exhibited DPPH radical scavenging activity, and induced the expression of tight junction (TJ) function-related proteins, as well as cell migration in HaCaT human keratinocytes. Additionally, we observed the inhibitory effects of AME on wrinkle formation by promoting collagen synthesis in CCD986Sk human fibroblast cells. This study represents the first evidence that *A. melanocarpa*, deemed safe and effective, possesses protective effects against damage in skin connective tissues. While our focus was on the potential use of AME in cosmetic agents, it's noteworthy that *A. melanocarpa* has traditional medicinal uses in Asia for treating various ailments. Our in vitro assays showcased novel cosmeceutical activities such as anti-wrinkle effects, suggesting that *A. melanocarpa*, a traditionally consumed herbal plant, could be incorporated into cosmeceutical products. This study contributes to expanding the application of traditional herbal foods into other industrial and scientific domains. For future investigations, we aim to identify more active compounds within AME and elucidate the detailed relationship between these compounds and the signaling pathways involved in collagen synthesis and TJ activation. In conclusion, our research may facilitate the development of new cosmetic and pharmaceutical substances utilizing *A. melanocarpa*.

Abbreviation

A. melanocarpa extract: AME; Dulbecco's modified Eagle's medium: DMEM; Fetal bovine serum: FBS; 1,1-diphenyl-2-picrylhydrazyl: DPPH; Ascorbic acid: A.A.; retinoic acid: R.A.; (1, 10)-Phenanthrolin: Phe; Elastatinal: Ela; Standard deviation: SD; Tight junction: TJ

Conflict of interest

The authors declare none of conflict of interest.

Consent for publications

All authors consented to writing this sentence, confirming that they have read and approved the final manuscript.

Ethics approval and consent to participate

The authors declare that they do not use humans or animals in their research.

Informed consent

Not available.

Availability of data and material

The authors declare that they embedded all data in the manuscript.

Authors' contribution

PSH: Conceptualization, Investigation, Supervision, Writing – original draft, review, and editing.

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References

1. Sidor A, Drożdżyńska A, Gramza-Michałowska A (2019) Black chokeberry (Aronia melanocarpa) and its products as potential health-promoting factors-An overview. Trends in Food Science & Technology 89: 45-60. doi:
2. Bijak M, Saluk J, Antosik A, Ponczek MB, Żbikowska HM, Borowiecka M, Nowak P (2013) Aronia melanocarpa as a protector against nitration of fibrinogen. International journal of biological macromolecules 55: 264-268. doi:
3. Bararu I, Badescu L, Badulescu O, Ciocoiu M, Badescu M (2013) Possibilities of limiting the inflammatory syndrome present in experimental diabetes mellitus by using natural poliphenols. Annals of the Romanian Society for Cell Biology 18 (2): 97. doi:
4. Cvetanović A, Zengin G, Zeković Z, Švarc-Gajić J, Ražić S, Damjanović A, Mašković P, Mitić M (2018) Comparative in vitro studies of the biological potential and chemical composition of stems, leaves and berries Aronia melanocarpa's extracts obtained by subcritical water extraction. Food and Chemical Toxicology 121: 458-466. doi:
5. Oprea E, Manolescu BN, Fărcăşanu IC, Mladin P, Mihele D (2014) Studies concerning antioxidant and hypoglycaemic activity of Aronia melanocarpa fruits. Revista Farmacia 62 (2): 254-263. doi:
6. Lee CM (2016) Fifty years of research and development of cosmeceuticals: a contemporary review. Journal of cosmetic dermatology 15 (4): 527-539. doi:
7. Matts PJ, Oblong JE, Bissett DL (2002) A review of the range of effects of niacinamide in human skin. Int Fed Soc Cosmet Chem Mag 5 (4): 285-289. doi:
8. Lee J, Ji J, Park SH (2018) Antiwrinkle and antimelanogenesis activity of the ethanol extracts of Lespedeza cuneata G. Don for development of the cosmeceutical ingredients. Food Science & Nutrition 6 (5): 1307-1316. doi:
9. Im DS, Lee J-M, Lee J, Shin HJ, No KT, Park S-H, Kim K (2017) Inhibition of collagenase and melanogenesis by ethanol extracts of Orostachys japonicus A. Berger: Possible involvement of Erk and Akt signaling pathways in melanoma cells. Acta biochimica et biophysica Sinica 49 (10): 945-953. doi:
10. Pinnell SR, Yang H, Omar M, Riviere NM, Debuys HV, Walker LC, Wang Y, Levine M (2001) Topical L-ascorbic acid: percutaneous absorption studies. Dermatologic surgery 27 (2): 137-142. doi:
11. Hori I, Nihei Ki, Kubo I (2004) Structural criteria for depigmenting mechanism of arbutin. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 18 (6): 475-479. doi:
12. Newton V, Mcconnell J, Hibbert S, Graham H, Watson R (2015) Skin aging: molecular pathology, dermal remodelling and the imaging revolution. G Ital Dermatol Venereol 150 (6): 665-674. doi:
13. Makrantonaki E, Zouboulis C (2007) Molecular mechanisms of skin aging: state of the art. Annals of the New York Academy of

Sciences 1119 (1): 40-50. doi:
14. Langton AK, Sherratt M, Griffiths C, Watson R (2010) A new wrinkle on old skin: the role of elastic fibres in skin ageing. International journal of cosmetic science 32 (5): 330-339. doi:
15. Shoulders MD, Raines RT (2009) Collagen structure and stability. Annual review of biochemistry 78: 929-958. doi:
16. Xu G-H, Ryoo I-J, Kim Y-H, Choo S-J, Yoo I-D (2009) Free radical scavenging and antielastase activities of flavonoids from the fruits of Thuja orientalis. Archives of pharmacal research 32 (2): 275-282. doi:
17. Apraj VD, Pandita NS (2016) Evaluation of skin anti-aging potential of Citrus reticulata blanco peel. Pharmacognosy research 8 (3): 160. doi:
18. Ravanti L, Heino J, López-Otín C, Kähäri V-M (1999) Induction of collagenase-3 (MMP-13) expression in human skin fibroblasts by three-dimensional collagen is mediated by p38 mitogen-activated protein kinase. Journal of Biological Chemistry 274 (4): 2446-2455. doi:
19. Bhagavathula N, DaSilva M, Aslam MN, Dame MK, Warner RL, Xu Y, Fisher GJ, Johnson KJ, Swartz R, Varani J (2009) Regulation of collagen turnover in human skin fibroblasts exposed to a gadolinium-based contrast agent. Investigative radiology 44 (8): 433. doi:
20. Her Y, Lee T-K, Kim JD, Kim B, Sim H, Lee J-C, Ahn JH, Park JH, Lee J-W, Hong J (2020) Topical application of Aronia melanocarpa extract rich in chlorogenic acid and rutin reduces UVB-induced skin damage via attenuating collagen disruption in mice. Molecules 25 (19): 4577. doi:
21. Lee H-R, Ryu HG, Lee Y, Park JA, Kim S, Lee CE, Jung S, Lee K-H (2022) Effect of Aronia Extract on Collagen Synthesis in Human Skin Cell and Dermal Equivalent. Oxidative Medicine and Cellular Longevity 2022. doi:
22. Kozłowska M, Brzóska MM, Rogalska J, Galicka A (2020) The impact of a polyphenol-rich extract from the berries of Aronia melanocarpa L. on collagen metabolism in the liver: A study in an in vivo model of human environmental exposure to cadmium. Nutrients 12 (9): 2766. doi:
23. Gelse K, Pöschl E, Aigner T (2003) Collagens—structure, function, and biosynthesis. Advanced drug delivery reviews 55 (12): 1531-1546. doi:
24. Di Lullo GA, Sweeney SM, Körkkö J, Ala-Kokko L, San Antonio JD (2002) Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. Journal of Biological Chemistry 277 (6): 4223-4231. doi:
25. Seo W-Y, Kim J-H, Baek D-S, Kim S-J, Kang S, Yang WS, Song J-A, Lee M-S, Kim S, Kim Y-S (2017) Production of recombinant human procollagen type I C-terminal propeptide and establishment of a sandwich ELISA for quantification. Scientific reports 7 (1): 15946. doi:
26. Lee H, Park JB, Ryu W-I, Kim JH, Shin JJ, Son SW (2016) Chloroform induces cystein-rich 61, a mediator of collagen homeostasis via early growth response-1 dependent pathway in human skin dermal fibroblasts. Molecular & Cellular Toxicology 12 (4): 337-343. doi:
27. Watanabe H, Shimizu T, Nishihira J, Abe R, Nakayama T, Taniguchi M, Sabe H, Ishibashi T, Shimizu H (2004) Ultraviolet A-induced production of matrix metalloproteinase-1 is mediated by macrophage migration inhibitory factor (MIF) in human dermal fibroblasts. Journal of Biological Chemistry 279 (3): 1676-1683. doi:
28. Meyer W, Neurand K, Radke B (1981) Elastic fibre arrangement in the skin of the pig. Archives of dermatological research 270 (4): 391-401. doi:
29. Brandner J (2007) Pores in the epidermis: aquaporins and tight junctions. International journal of cosmetic science 29 (6): 413-422. doi:
30. Volksdorf T, Heilmann J, Eming SA, Schawjinski K, Zorn-Kruppa M, Ueck C, Vidal-y-Sy S, Windhorst S, Jücker M, Moll I (2017) Tight Junction Proteins Claudin-1 and Occludin Are Important for Cutaneous Wound Healing. The American Journal of Pathology 187 (6): 1301-1312. doi: