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The Role of Anthocyanin-Rich Phytoestrogens in Collagen Synthesis and Tissue Regeneration: Histological and Cellular Insights : *A Comprehensive Systematic Review*

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ABSTRACT

Background: Tissue regeneration, collagen synthesis, and vascular health decline with age and hormonal changes. Recently, anthocyanin-rich phytoestrogens have emerged as natural alternatives to support these processes by mimicking estrogen. This systematic review aims to provide a comprehensive overview on the potential of anthocyanin-rich phytoestrogens as natural agents for promoting collagen stimulation and tissue regeneration. **Methods:** The study followed PRISMA 2020 guidelines, reviewing English-language publications from 2014 to 2024. Editorials, duplicate reviews from the same journal, and papers lacking a DOI were excluded. The literature search was conducted using PubMed, SagePub, SpringerLink, and Google Scholar. **Result:** A total of 2,172 articles were initially identified through online databases (PubMed, SagePub, SpringerLink, and Google Scholar). After three rounds of screening, eight relevant studies were selected for full-text analysis. **Conclusion:** Anthocyanin-rich phytoestrogens show promise in enhancing ECM stability and tissue regeneration via estrogen receptor modulation. Future clinical trials are needed to validate their effects, determine safe dosing, and explore synergistic combinations for optimal therapeutic use in estrogen-deficient conditions..

Keyword: Anthocyanin, phytoestrogen, collagen stimulation, tissue regeneration

INTRODUCTION

Tissue regeneration and collagen synthesis are central to maintaining skin elasticity, bone density, and vascular health, yet these functions often diminish with age and hormonal changes. Estrogen plays a key role in regulating extracellular matrix (ECM) integrity and collagen formation, which decline significantly during menopause, leading to structural weaknesses in various tissues.[1–4]

In recent years, anthocyanin-rich phytoestrogens—compounds derived from plants that can mimic estrogen—have emerged as potential natural alternatives for supporting tissue regeneration. These compounds, abundant in foods like blackcurrant and Hibiscus sabdariffa, are believed to stimulate estrogen receptors and influence pathways associated with ECM stability. However, the extent and mechanisms by which anthocyanins affect tissue health and regeneration remain under exploration.[5–9]

Interest in anthocyanins as a therapeutic option is largely driven by their unique molecular structure, allowing them to interact with specific estrogen receptors, particularly ERβ. Studies suggest that anthocyanins have a greater affinity for ERβ, which plays a protective role in numerous tissues, including skin, bones, and the brain.[10,11] This receptor selectivity could make anthocyanins safer and more targeted alternatives to conventional hormone replacement therapy (HRT), which often activates both ERα and ERβ, leading to unintended side effects. Consequently, research into these compounds offers exciting potential for safe, effective therapies that leverage natural estrogenic pathways without the risks associated with synthetic hormones.[12–14]

The unique anti-inflammatory and antioxidant properties of anthocyanins may also contribute to their role in tissue health. In conditions of chronic inflammation or oxidative stress, tissues often experience accelerated collagen breakdown and compromised ECM integrity, leading to early onset of skin aging, bone fragility, and vascular stiffness. Anthocyanins have shown potential in reducing systemic inflammation and oxidative damage, which could indirectly support ECM maintenance and regeneration in aging tissues.[15–17]

Despite their potential, the effects of anthocyanin-rich phytoestrogens on ECM dynamics and tissue regeneration have only recently begun to receive systematic attention. While initial studies show promising outcomes in animal models, such as enhanced collagen production, reduced bone density loss, and improved skin elasticity, further investigation is necessary to determine the specific molecular pathways involved. [18–20]

This systematic review aims to provide a comprehensive overview on the potential of anthocyanin-rich phytoestrogens as natural agents for promoting collagen stimulation and tissue regeneration.

METHODS

Protocol

The study strictly adhered to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines to ensure methodological rigor and accuracy. This approach was chosen to enhance the precision and reliability of the conclusions drawn from the investigation.

Criteria for Eligibility

This systematic review aims to provide a comprehensive overview on the potential of anthocyanin-rich phytoestrogens as natural agents for promoting collagen stimulation and tissue regeneration based on literature from the past decade. The review aimed to provide insights to improve patient treatment strategies, with an emphasis on the significance of key findings in the reviewed studies. Inclusion criteria for the study included: 1) Papers published in English, and 2) Papers published between 2014 and 2024. Exclusion criteria were: 1) Editorials, 2) Papers without a DOI, 3) Previously published review articles, and 4) Duplicate entries in journals..

Search Strategy

The keywords used for this research are Anthocyanin, phytoestrogen, collagen stimulation, tissue regeneration. The Boolean MeSH keywords inputted on databases for this research are: *((("anthocyanine"[All Fields] OR* *"anthocyanines"[All Fields] OR "anthocyanins"[MeSH Terms] OR "anthocyanins"[All Fields] OR "anthocyanin"[All Fields]) AND ("phytoestrogenes"[All Fields] OR "phytoestrogenic"[All Fields] OR "phytoestrogens"[Pharmacological Action] OR "phytoestrogens"[MeSH Terms] OR "phytoestrogens"[All Fields] OR "phytoestrogen"[All Fields]) AND (("collagen"[MeSH Terms] OR "collagen"[All Fields] OR "collagens"[All Fields] OR "collagen s"[All Fields] OR "collagenation"[All Fields] OR "collagene"[All Fields] OR "collageneous"[All Fields] OR "collagenic"[All Fields] OR "collagenization"[All Fields] OR "collagenized"[All Fields] OR "collagenous"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields]))) OR (("tissue s"[All Fields] OR "tissues"[MeSH Terms] OR "tissues"[All Fields] OR "tissue"[All Fields]) AND ("regenerability"[All Fields] OR "regenerable"[All Fields] OR "regenerant"[All Fields] OR "regenerants"[All Fields] OR "regenerate"[All Fields] OR "regenerated"[All Fields] OR "regenerates"[All Fields] OR "regenerating"[All Fields] OR "regeneration"[MeSH Terms] OR "regeneration"[All Fields] OR "regenerations"[All Fields]))) AND (y_10[Filter])*

Data retrieval

Abstracts and titles were screened to assess their eligibility, and only studies meeting the inclusion criteria were selected for further analysis. Literature that fulfilled all predefined criteria and directly related to the topic was included. Studies that did not meet these criteria were excluded. Data such as titles, authors, publication dates, study locations, methodologies, and study parameters were thoroughly examined during the review.

Quality Assessment and Data Synthesis

Each author independently assessed the titles and abstracts of the selected studies to identify those for further exploration. Articles that met the inclusion criteria underwent further evaluation. Final decisions on inclusion were based on the findings from this review process.

Table 1. Article Search Strategy

Database	Keywords	Hits
Pubmed	((("anthocyanine"[All Fields] OR "anthocyanines"[All Fields] OR "anthocyanins"[MeSH Terms] OR "anthocyanins"[All Fields] OR "anthocyanin"[All Fields]) AND ("phytoestrogenes"[All Fields] OR "phytoestrogenic"[All Fields] OR "phytoestrogens"[Pharmacological Action] OR "phytoestrogens"[MeSH Terms] OR "phytoestrogens"[All Fields] OR "phytoestrogen"[All Fields]) AND (("collagen"[MeSH Terms] OR "collagen"[All Fields] OR "collagens"[All Fields] OR "collagen s"[All Fields] OR "collagenation"[All Fields] OR "collagene"[All Fields] OR "collageneous"[All Fields] OR "collagenic"[All Fields] OR "collagenization"[All Fields] OR "collagenized"[All Fields] OR "collagenous"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields]))) OR (("tissue s"[All Fields] OR "tissues"[MeSH Terms] OR "tissues"[All Fields] OR "tissue"[All Fields]) AND ("regenerability"[All Fields] OR "regenerable"[All Fields] OR "regenerant"[All Fields] OR "regenerants"[All Fields] OR "regenerate"[All Fields] OR "regenerated"[All Fields] OR "regenerates"[All Fields] OR "regenerating"[All Fields] OR "regeneration"[MeSH Terms] OR "regeneration"[All Fields] OR "regenerations"[All Fields]))) AND (y_10[Filter])	466
Springer Link	(((Anthocyanin) AND (phytoestrogen)) AND (collagen stimulation)) OR (tissue regeneration)	402
Sagepub	(((Anthocyanin) AND (phytoestrogen)) AND (collagen stimulation)) OR (tissue regeneration)	500
Google Scholar	(((Anthocyanin) AND (phytoestrogen)) AND (collagen stimulation)) OR (tissue regeneration)	804

Table 2. JBI *Critical appraisal of Study*

Parameters	Nanashima (2018)	Lorenzana-Martinez (2023)	Nanashima (2015)	Horie (2021)	Nanashima (2017)	Zheng (2016)	Shimada (2022)	Benn (2015)
1. Bias related to temporal precedence								
Is it clear in the study what is the "cause" and what is the "effect" (ie, there is no confusion about which variable comes first)?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Bias related to selection and allocation								
Was there a control group?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3. Bias related to confounding factors								
Were participants included in any comparisons similar?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4. Bias related to administration of intervention/exposure								
Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	No.	No.	No.	No.	No.	No.	No.	No.
5. Bias related to assessment, detection, and measurement of the outcome								
Were there multiple measurements of the outcome, both pre and post the intervention/exposure?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were the outcomes of participants included in any comparisons measured in the same way?	No.	No.	No.	No.	No.	No.	No.	No.
Were outcomes measured in a reliable way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6. Bias related to participant retention								
Was follow-up complete and, if not, were differences between groups in terms of their follow-up adequately described and analyzed?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7. Statistical conclusion validity								
Was appropriate statistical analysis used?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

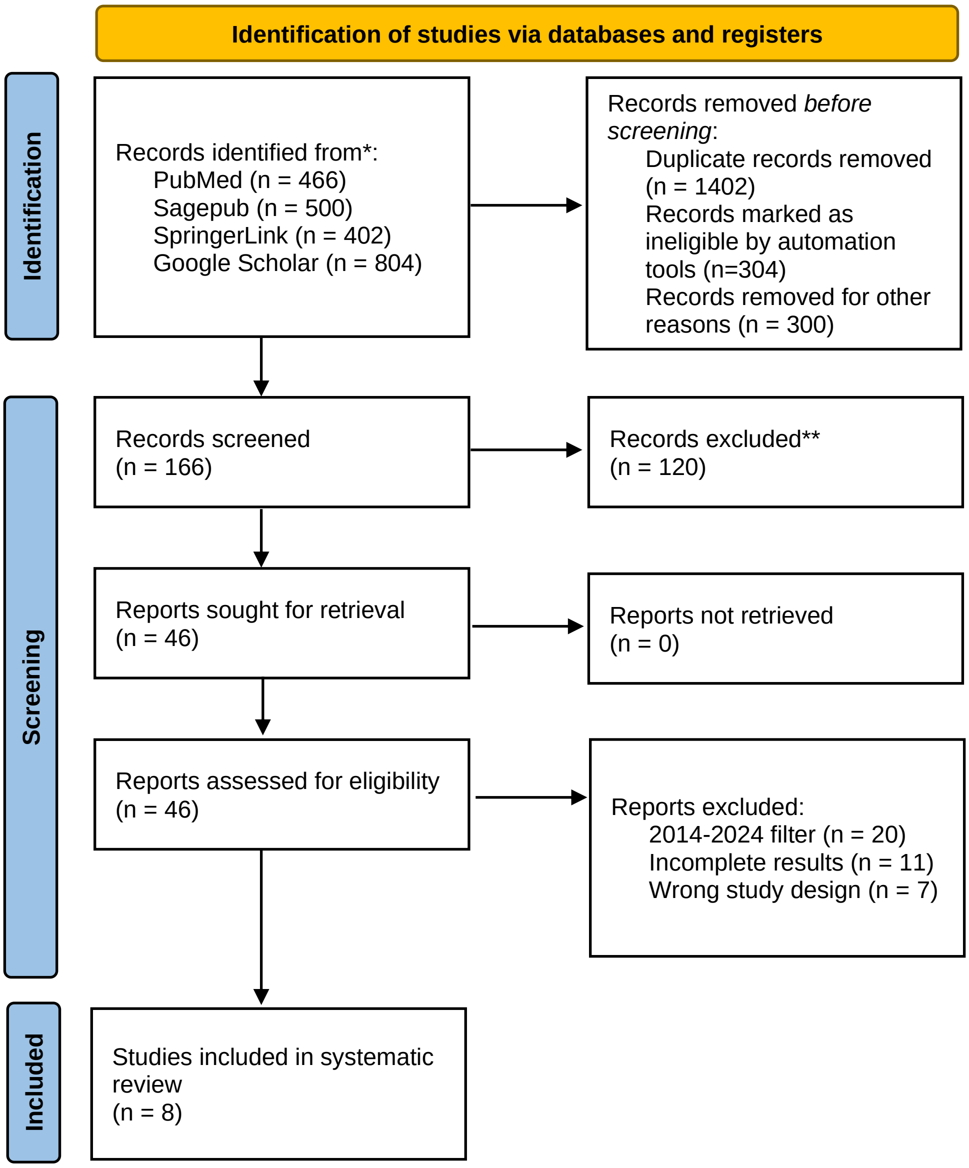

Figure 1. Article search flowchar

RESULT

The initial number of articles retrieved from online databases (PubMed, SagePub, SpringerLink, and Google Scholar) is 2,172 articles. After conducting three levels of screening, eight articles that directly relate to the current systematic review have been chosen for further assessment through full-text reading and analysis. Table 1 presents the selected literature included in this analysis.

Table 1. The literature included in this study

No.	Author	Origin	Method	Sample	Result
1.	Nanashima, et al.[21] (2018)	Japan	In vivo study	10 samples	In TIG113 cells, microarray and Ingenuity® Pathway Analysis revealed that 1.0 µg/mL of blackcurrant extract (BCE) upregulated numerous estrogen-related genes. Quantitative RT-PCR confirmed that BCE (1.0 or 10.0 µg/mL) and four types of anthocyanins (10 µM) modified mRNA expression of extracellular matrix (ECM) proteins and enzymes involved in ECM turnover. Immunofluorescence staining showed that anthocyanins promoted the expression of ECM proteins, including collagen (types I and III) and elastin. Additionally, a 3% dietary administration of BCE to ovariectomized rats over 3 months increased skin collagen, elastin, and hyaluronic acid.
2.	Lorenzana-Martinez, et al.[22] (2023)	Mexico	In vivo study	30 samples	In a Wistar rat model of ovariectomy (OVX), recent findings demonstrate that an anthocyanin-rich extract from Hibiscus sabdariffa (HSE) helps mitigate memory deficits associated with estrogen deficiency and may exert phytoestrogenic effects by modulating estrogen receptor (ER) expression. Specifically, HSE influenced ERα expression more strongly, while

					estradiol primarily affected ERβ.
3.	Nanashima, et al.[23] (2015)	Japan	In vivo study	15 samples	Microarray and Ingenuity® Pathway Analysis revealed that blackcurrant extract (BCE) activates the estrogen receptor beta (ERβ) pathway, with quantitative PCR confirming that BCE and anthocyanins upregulated ERβ downstream genes. BCE (0.1–1.0 μg/mL) and anthocyanins (0.1–10 μM) promoted MCF-7 cell proliferation, an effect blocked by the ER antagonist fulvestrant. Flow cytometry showed that anthocyanins altered cell cycle distribution, decreasing cells in G0/G1 and increasing those in G2/M phases. Anthocyanins also stimulated ERβ activity in ERβ reporter assays and increased alkaline phosphatase activity in Ishikawa cells. Competition assays and in silico analysis suggested that anthocyanins can bind to ERβ.
4.	Horie, et al.[24] (2021)	Japan	In vivo study	10 samples	The study found that ovariectomized (OVX) control rats had lower staining intensity of elastic fibers, increased elastin fragmentation, and elevated α-smooth muscle actin expression compared to OVX rats treated with blackcurrant extract (BCE) and sham-operated rats. Pathological vascular remodeling was observed

					only in OVX control rats. Additionally, matrix metalloproteinase-12 (MMP-12) mRNA levels, associated with elastin degradation, were significantly higher in OVX control rats than in the sham and OVX BCE groups.
5.	Nanashima, et al.[25] (2017)	Japan	In vivo study	10 samples	Blackcurrant anthocyanins activate ERβ-mediated transcription with higher binding affinity for ERβ over ERα, as indicated by a lower IC50 for ERβ. In silico docking analysis of anthocyanins' core components, cyanidin and delphinidin, showed hydrogen bond formation with ERβ residues (Glu305, Arg346, and His475), similar to 17β-estradiol. While glucoside or rutinoside sugar chains did not fit into ERβ's ligand-binding pocket, structural flexibility in ERβ helices may allow accommodation of these chains. Comparison with ERα confirmed greater selectivity for ERβ, akin to genistein.
6.	Zheng, et al.[26] (2016)	USA	In vivo study	54 samples	Ovariectomy significantly decreased bone mineral density (BMD) and trabecular bone volume in mice. Blackcurrant supplementation, however, helped reduce this bone loss, with effects becoming more notable at 12 weeks. Despite no changes in serum bone formation and

					resorption biomarkers, blackcurrant treatment reduced osteoclast-like (OCL) cell formation and the number of TRAP(+) OCL cells in ovariectomized mice. Additionally, blackcurrant supplementation decreased bone resorption activity in resorption pit assays.
7.	Shimada, et al.[27] (2022)	Japan	In vivo study	10 samples	A 9-week dietary supplementation with anthocyanin-rich blackcurrant extract (BCE) improved long-term recognition memory and normalized anxiety in SAMP8 mice, a model with Alzheimer's-like features. RNA sequencing showed that BCE significantly altered hippocampal gene expression, affecting genes associated with the smooth endoplasmic reticulum, axons, and glutamatergic synapses. Real-time PCR confirmed changes in Alzheimer's disease (AD)-related gene expression.
8.	Benn, et al.[28] (2015)	USA	In vivo study	11 samples	In mice, blackcurrant extract (BCE) supplementation significantly reduced plasma total cholesterol (TC) and glucose levels, while plasma triglycerides (TAG) remained unchanged. BCE also decreased hepatic TAG levels and liver steatosis. Although BCE did not significantly alter mRNA levels of key regulators in cholesterol metabolism,

					such as HMGR and LDLR, it did increase protein expression of LDLR and mature sterol-regulatory element-binding protein. Additionally, BCE lowered levels of proprotein convertase subtilisin/kexin type 9 (PCSK9), which degrades LDLR, and its regulator, hepatocyte nuclear factor 4α.

Nanashima, et al.[21] (2018) provides strong evidence that blackcurrant phytoestrogens, particularly anthocyanins, positively impact skin health by enhancing ECM protein expression, including collagen and elastin.

Lorenzana-Martinez, et al.[22] (2023) suggest H. sabdariffa could be a potential nutrition-based alternative to hormone replacement therapy (HRT), warranting further investigation into its applications for managing estrogen-deficient conditions.

Nanashima, et al.[23] (2015) showed BCE induced stratification of uterine columnar epithelial cells and elevated cytoplasmic mucin levels. These findings indicate that blackcurrant anthocyanins function as phytoestrogens both in vitro and in vivo.

Horie, et al.[24] (2021) suggest that BCE provides vascular protection by reducing MMP-12 expression and vascular smooth muscle cell proliferation, marking the first evidence that BCE may prevent elastin degradation and vascular remodeling during menopause.

Nanashima, et al.[25] (2017) findings highlight the phytoestrogenic activity of blackcurrant anthocyanins via ERβ.

Zheng, et al.[26] (2016) suggest that blackcurrant may be effective in mitigating osteoclast-driven bone loss associated with postmenopausal osteoporosis.

Shimada, et al.[27] (2022) suggest that anthocyanin-rich BCE may offer potential for AD prevention or treatment, making it a promising dietary supplement for cognitive health.

Benn, et al.[28] (2015) suggest BCE may help prevent metabolic issues associated with high-fat, high-cholesterol diets.

DISCUSSION

Anthocyanin-rich phytoestrogens, derived from sources like blackcurrant and Hibiscus sabdariffa, have gained attention for their potential to enhance collagen synthesis and modulate extracellular matrix (ECM) turnover, crucial for maintaining skin and tissue health. Phytoestrogens, compounds with estrogen-like effects, interact with estrogen receptors (ERs) to influence pathways involved in tissue regeneration. [29,30]

Nanashima et al. (2018) demonstrated that blackcurrant extract (BCE) upregulates collagen types I and III as well as elastin, showing a strong effect on ECM protein expression. This study suggests that anthocyanins may play a critical role in addressing age-related collagen degradation, particularly in estrogen-deficient individuals, making them a promising alternative to traditional treatments.[21]

Mechanistically, the estrogen receptor-mediated pathway is central to ECM protein regulation. Studies by Nanashima et al. (2015, 2017) show that BCE's impact on ERβ pathways promotes downstream gene expression related to ECM protein production, supporting collagen synthesis and cellular proliferation. These findings highlight BCE's selective affinity for ERβ over ERα, with lower IC50 values indicating a more robust activation of ERβ. This selective action is significant in managing ECM degradation associated with estrogen decline, suggesting that targeting ERβ specifically could provide therapeutic benefits without the adverse effects associated with broader estrogenic stimulation.[23,25]

Histological findings further illustrate BCE's positive effects on ECM integrity. Horie et al. (2021) reported that BCE treatment reduced elastin degradation in vascular tissues of ovariectomized (OVX) rats, indicating a protective effect against aging-related vascular remodeling. BCE-treated rats displayed lower MMP-12 expression, an enzyme associated with elastin degradation, and improved vascular morphology. These findings suggest BCE's capability to preserve ECM structure, offering protective benefits against age-related deterioration of both vascular and dermal tissues.[24]

When comparing the effects of blackcurrant and Hibiscus sabdariffa extracts, their differential impact on ER expression is notable. Lorenzana-Martinez et al. (2023) found that H. sabdariffa extract (HSE) had a stronger influence on ERα expression, while BCE predominantly targeted ERβ pathways. This differential receptor targeting implies that combining both extracts may produce complementary phytoestrogenic effects, potentially benefiting a broader range of tissues involved in ECM maintenance and repair. This approach could create a more comprehensive alternative for managing ECM degradation in estrogen-deficient conditions, particularly postmenopausal tissue degeneration.[22]

The positive effects of anthocyanins extend beyond ECM stability to bone health. Zheng et al. (2016) found that blackcurrant supplementation reduced bone loss in OVX-induced models, attributed to decreased osteoclastic activity and bone resorption markers. Although anthocyanins did not alter serum biomarkers, they mitigated osteoclast formation, indicating that these compounds not only support ECM integrity in soft tissues but may also reduce bone density loss, which is common in postmenopausal osteoporosis. These findings underscore anthocyanins' broad potential in tissue regeneration, encompassing both soft and hard tissues.[30]

In addition to ECM regulation, anthocyanin-rich BCE has shown promise in cognitive health, with potential implications for neural ECM integrity. Shimada et al. (2022) found that BCE supplementation improved memory retention and reduced anxiety in SAMP8 mice, a model of Alzheimer's-like conditions. Gene expression analyses revealed that BCE influenced hippocampal genes linked to the smooth endoplasmic reticulum, axonal stability, and synaptic function. This suggests that BCE's neuroprotective effects may extend to ECM stability in neural tissue, providing another dimension of health benefits related to cognitive decline.[27]

Molecular docking analyses further clarify the mechanisms underlying BCE's effects. Nanashima et al. (2017) used in silico docking studies to show that blackcurrant anthocyanins, such as cyanidin and delphinidin, bind to key residues within ERβ, with interactions similar to 17β-estradiol. This binding specificity for ERβ over ERα aligns with BCE's selective action on ERβ pathways observed in vitro. Such findings emphasize the potential to design targeted therapies based on the molecular structures of anthocyanins, optimizing their impact on ERβ and enhancing tissue regeneration without broader estrogenic effects.[31]

Beyond ECM synthesis, the metabolic effects of BCE also indirectly support ECM stability. Benn et al. (2015) showed that BCE supplementation reduced plasma cholesterol and glucose levels while decreasing hepatic steatosis. Although these metabolic changes do not directly impact ECM synthesis, they may reduce systemic inflammation, a factor that can destabilize ECM in tissues vulnerable to metabolic stress, such as vascular and dermal structures. This anti-inflammatory effect could make BCE a valuable supplement for conditions where both metabolic and ECM health are compromised.[28]

The phytoestrogenic actions of blackcurrant and H. sabdariffa suggest a potential alternative to hormone replacement therapy (HRT) for estrogen-deficient conditions. As Lorenzana-Martinez et al. (2023) discussed, anthocyanin-rich phytoestrogens could provide a safer, nutrition-based option for managing symptoms related to skin aging, vascular remodeling, and osteoporosis. Such alternatives are particularly relevant in cases where traditional HRT poses risks, offering the benefits of estrogenic stimulation without the associated side effects.[22]

CONCLUSION

Anthocyanin-rich phytoestrogens show promise in enhancing ECM stability and tissue regeneration via estrogen receptor modulation. Future clinical trials are needed to validate their effects, determine safe dosing, and explore synergistic combinations for optimal therapeutic use in estrogen-deficient conditions..

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