AJPR
Avicenna Journal of Pharmaceutical Research
Avicenna J Pharma Res. 2025 ;6(1):59-70
DOI: 10.53208/ajpr.6.1.59
http://ajpr.umsha.ac.ir



Original Article

Natural Carotenoids Targeting Matrix Metalloproteinase-3: Implications for Caries Prevention

Farnaz Monsef [1], Fatemeh Khedmatloo[2], Amir Taherkhani[3, 4*]

1. Department of Restorative Dentistry, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
2. Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran
3. Research Center for Molecular Medicine, Institute of Cancer, Hamadan University of Medical Sciences, Hamadan, Iran
4. Urology and Nephrology Research Center, Avicenna Institute of Clinical Sciences, Hamadan University of Medical Sciences, Hamadan, Iran

Article history:
Received: May 14, 2025
Revised: May 23, 2025
Accepted: May 26, 2025
Published: July 27, 2025

***Corresponding author:**
Amir Taherkhani, Research Center for Molecular Medicine, Institute of Cancer, Hamadan University of Medical Sciences, Hamadan, Iran
Email: amir.007.taherkhani@gmail.com

Abstract

Background: Dental caries, a pervasive chronic disease, arises from a complex interplay of cariogenic bacteria, dietary sugars, and host factors. Matrix metalloproteinase-3 (MMP-3), an enzyme detected in demineralized human dentin and bovine predentine, contributes to caries progression through its proteoglycan-degrading activity. This study aimed to evaluate a series of carotenoids for their potential to inhibit the MMP-3 active site.

Methods: A molecular docking analysis was performed using AutoDock 4.0 to estimate the binding affinity of 19 carotenoid compounds to the catalytic cleft of MMP-3. The specific binding modes and molecular interactions were subsequently analyzed and visualized using the Discovery Studio Visualizer. The results were compared with those of a positive control inhibitor, MBS.

Results: Three carotenoids, including astaxanthin, canthaxanthin, and capsanthin, displayed notably strong binding affinities, with free energy values ($\Delta G_{binding}$) lower than -10 kcal/mol. Among these, astaxanthin was the most potent inhibitor. Interaction profiling indicated that capsanthin established the most extensive hydrophobic network, engaging five key residues, while canthaxanthin formed two stabilizing hydrogen bonds.

Conclusion: The findings suggest that astaxanthin, canthaxanthin, and capsanthin are promising natural compounds for inhibiting MMP-3. This positions them as potential candidates for novel therapeutic strategies to prevent dental caries progression.

Keywords: Carotenoids, Dental caries, Drug, Inhibitor, Matrix metalloproteinase-3

Introduction

Dental caries is a global chronic health issue stemming from the complex, long-term interactions among acid-producing bacteria, fermentable carbohydrates, and individual patient factors [1]. According to the World Health Organization, oral diseases affect approximately 3.5 billion people, and untreated tooth decay alone is the most common health problem on a global scale [2]. This condition is highly prevalent in children, impacting 43% worldwide [3, 4]. Despite modern advances in dentistry, the burden of caries remains substantial, particularly in developing countries and among at-risk populations [5]. Therefore, early detection of cavities and preventive strategies are vital for controlling disease progression and preserving natural teeth [6].

The pathogenesis of dental caries involves a shift in microbial communities within oral biofilms. The primary mechanism involves cariogenic bacteria, notably *Streptococcus mutans* (*S. mutans*), metabolizing fermentable carbohydrates in plaque

© 2025 The Author(s); Published by Hamadan University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

biofilms to generate acids [7]. This leads to dysbiosis, reinforcing *S. mutans* dominance and creating persistent acidic microenvironments that demineralize enamel and dentin, thereby initiating caries [8, 9]. In addition to bacterial acids, the degradation of the dentinal matrix is facilitated by host-derived enzymes, specifically matrix metalloproteinases (MMPs), which degrade collagen fibrils. The clinical relevance of this process is evidenced by the use of MMP inhibitors to pretreat dentin before restorative bonding [10]. Among these enzymes, MMP-3 has been identified in demineralized human dentin, where it acts as a proteoglycanase. Its specific proteolytic activity in this context is well-documented, and its presence has been further confirmed in the predentine layer and dentinal tubules of bovine molars [11-14].

Carotenoids are widely distributed pigments responsible for yellow, orange, and red hues. Plants, bacteria, and fungi biosynthesize these C40 tetraterpenoids, but animals do not produce them and must obtain them through nutrition. Their medicinal appeal stems from a range of biological functions, including scavenging free radicals, exerting anti-inflammatory and provitamin A activities, and displaying anti-aging properties [15-20].

Carotenoids have shown inhibitory effects against MMPs. For example, a previous study by Sajjad et al. [21] demonstrated through molecular docking that astaxanthin exhibits a high binding affinity for MMP-3, interacting with its hydrophobic S1' active site. Building on this finding, the present study aimed to evaluate the inhibitory potential of a diverse set of carotenoids against MMP-3 using AutoDock 4.0 and to critically compare their binding modes and affinities with those of the potent MMP-3 inhibitor (MBS; PubChem ID: 446165). This comparative investigation identified novel, plant-derived MMP-3 inhibitors, providing a foundational rationale for developing herbal-based interventions to combat collagen degradation in dentin and help prevent dental caries.

Materials and Methods

Re-docking Analysis

Prior to carotenoid screening, the docking protocol was validated by re-docking MBS (the co-crystallized ligand from MMP-3, PDB ID: 1HY7) using the same AutoDock 4.0 settings. Root-mean-square deviation (RMSD) between the re-docked and crystal poses was calculated using LigRMSD [22] to confirm binding mode reproducibility.

Matrix Metalloproteinase-3 (MMP-3) and Carotenoids Structural Preparation

To establish a high-resolution structural framework for this investigation, the crystal structure of MMP-3 (PDB ID: 1HY7) was retrieved from the RCSB Protein Data Bank [23, 24]. This structure, determined at 1.5 Å resolution via X-ray crystallography, features two polypeptide chains. In accordance with our earlier work [14], Chain B (residues 583-755) was chosen as the model for all subsequent analyses. The initial protein preparation involved excising all non-protein atoms, ions, and the co-crystallized inhibitor MBS. The DSV tool was employed to characterize the binding pose of the native MBS inhibitor, thereby defining the critical residues within the enzymatic active site. The protein was then energy-minimized in Swiss-PdbViewer to obtain a stable conformation [25]. For the docking studies, a collection of nineteen distinct carotenoids (β-Carotene, Lutein, Astaxanthin, Bixin, Norbixin, Capsanthin, Lycopene, Canthaxanthin, Zeaxanthin, Peridinin, Fucoxanthin, β-Cryptoxanthin, Violaxanthin, Antheraxanthin, Diadinoxanthin, Diatoxanthin, α-Carotene, Crocetin, and Neoxanthin), along with the control inhibitor MBS, was assembled.

Energy minimization of the ligands was performed using HyperChem [26], employing molecular mechanics optimization with the Polak-Ribiere algorithm and a root-mean-square gradient of 0.1 kcal/(Å·mol). The potential of these compounds as inhibitors was assessed by comparing their binding affinities at the MMP-3 active site with those of MBS.

Molecular Docking

The computational docking procedure was conducted as follows. All calculations were performed on a workstation featuring an Intel Core i7 CPU, 24 GB of RAM, and a 64-bit Windows operating system. Using AutoDock 4.0 [27], a semi-

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

flexible docking approach was employed to estimate the binding free energy ($\Delta G_{binding}$) of the ligands to the MMP-3 catalytic site. A grid box was centered on the active site (coordinates: 0.231, 49.256, 53.451 Å) with dimensions of 52 × 48 × 74 Å to encompass the 18 key residues identified in a prior analysis [14]. For each compound, 50 independent docking runs were performed using the Lamarckian Genetic Algorithm. The resulting poses were clustered using a 2.0 Å RMSD threshold. The pose exhibiting the most negative $\Delta G_{binding}$ value within the most significant cluster was chosen for detailed evaluation and visualization; the latter was performed using the DSV tool.

Interaction Modes

To identify the most effective MMP-3 inhibitors, a selection was made by evaluating $\Delta G_{binding}$ values. Specifically, compounds with binding energies greater than −10 kcal/mol were classified as high-affinity binders and shortlisted. The molecular interactions of these shortlisted compounds were then visualized and analyzed using the DSV tool, and their binding profiles were directly compared with those of the control inhibitor.

Zinc-Inclusive Docking Validation

Docking was repeated with the zinc ion retained in 1HY7 for the top-three compounds and MBS, using the same parameters as the zinc-free protocol: grid box (52 × 48 × 74 Å), center (0.231, 49.256, 53.451 Å), 50 runs, and AutoDock 4.0's Lamarckian Genetic Algorithm. Binding energies and interactions were compared to evaluate the zinc ion's role in binding affinity and mode.

Results

Re-docking Validation

MBS was successfully re-docked into 1HY7, yielding a strict RMSD of 2.43 Å (Figure 1). This falls within the 2.5 Å cutoff, confirming the reliability of the AutoDock 4.0 protocol for the carotenoid library. Although an RMSD of 2.0 Å is generally accepted as the standard threshold [28], values up to 2.5 Å are tolerated for ligands with numerous dihedral degrees of freedom [29].

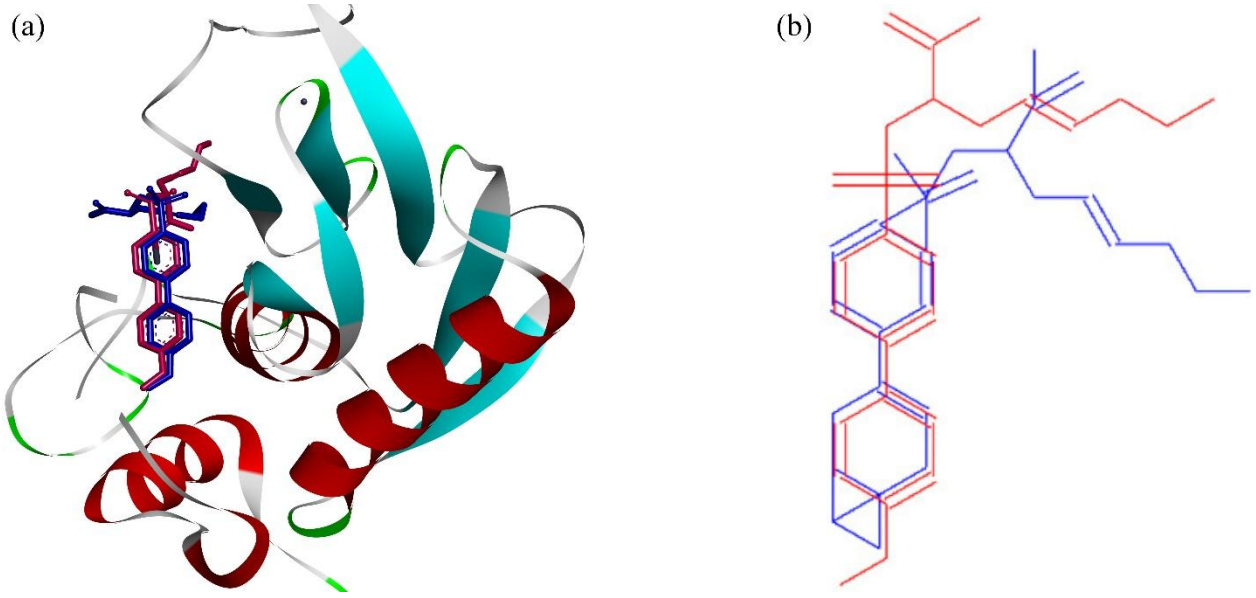


Figure 1. Validation of the Molecular Docking Analysis, (a) Superimposition of the Crystallographic (red) and Re-docked (blue) Conformations of MBS within the 1HY7 Catalytic Domain, Visualized Using Discovery Studio Visualizer, (b) Two-Dimensional Superposition of the Isolated Ligand Posing Generated by LigRMSD, Illustrating Close Agreement Between the Experimental and Predicted Binding Modes, with a Calculated Root-Mean-Square Deviation (RMSD) of 2.43 Å.

Gibbs Free Energy of Binding Assessment

This study identified the most promising inhibitors for the target by calculating their $\Delta G_{binding}$ values to the catalytic site. Several carotenoids showed significant potential, with binding energies below −10 kcal/mol. Specifically, astaxanthin,

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

canthaxanthin, and capsanthin demonstrated strong inhibitory effects, with binding energies of −10.66, −10.58, and −10.15 kcal/mol, respectively. Their potency was reflected in nanomolar inhibition constants (Ki) of 15.24 nM, 17.58 nM, and 36.01 nM. For comparison, the control compound MBS was less potent, with a $\Delta G_{binding}$ of -9.34 kcal/mol and a Ki value in the picomolar range (142.89 nM). Table 1 presents a complete breakdown of all calculated energy types and Ki values for every tested ligand. Figure 2 shows a comparison of the estimated Gibbs free binding energies for the tested ligands interacting with the 1HY7 catalytic cleft.

Docking Validation with Internal Zinc Ion

The inclusion of zinc ions caused only minor changes in the binding energies of the tested compounds, indicating that the catalytic zinc contributes minimally to ligand coordination within the active site. The compound ranking remained unchanged, with astaxanthin showing the most favorable binding energy, followed by canthaxanthin, capsanthin, and MBS (Table 2).

Table 1. Details of Energies and Ki Values Between the Tested Compounds in This Study and the MMP-3 Active Site, in the Absence of Zinc Ions

PubChem ID	Name	Gibbs Free Binding Energy (kcal/mol)	Unbound System's Energy (kcal/mol)	Torsional Free Energy (kcal/mol)	Final Total Internal Energy (kcal/mol)	Final Intermolecular Energy (kcal/mol)	Ki
5281224	Astaxanthin	-10.66	-1.63	6.26	-4.72	-13.84	15.24 nM
5281227	Canthaxanthin	-10.58	-1.59	5.67	-4.72	-13.12	17.58 nM
5281228	Capsanthin	-10.15	-1.38	6.56	-4.97	-13.13	36.01 nM
5280489	β-Carotene	-9.95	-1.52	5.67	-4.23	-12.91	50.73 nM
6440986	Diatoxanthin	-9.84	-1.5	6.26	-4.56	-13.04	61.77 nM
5289155	Peridinin	-9.72	-1.71	5.67	-3.17	-13.92	74.78 nM
5281223	Antheraxanthin	-9.57	-1.47	6.26	-4.3	-13	97.32 nM
5281239	Fucoxanthin	-9.46	-1.99	6.86	-3.93	-14.38	116.63 nM
5281232	Crocetin	-9.44	-1.52	5.67	-3.83	-12.8	120.78 nM
5281235	β-Cryptoxanthin	-9.3	-1.5	5.97	-4.52	-12.25	152.36 nM
5280899	Zeaxanthin	-9.07	-1.49	6.26	-3.42	-13.41	224.09 nM
5281243	Lutein	-8.84	-1.48	6.26	-4.87	-11.72	331.03 nM
448438	Violaxanthin	-8.75	-1.49	6.26	-2.86	-13.64	384.59 nM
6449888	Diadinoxanthin	-8.33	-1.49	6.26	-4.54	-11.55	781.79 nM
5282217	Neoxanthin	-8	-1.5	6.56	-4.04	-12.02	1.37 uM
5281226	Bixin	-7.88	-0.77	6.26	-2.26	-12.65	1.68 uM
446925	Lycopene	-7.71	-1.37	8.65	-5.82	-11.91	2.22 uM
5281249	Norbixin	-7.07	-0.64	6.26	-1.15	-12.83	6.59 uM
4369188	α-Carotene	-5.12	-0.51	5.07	-2.22	-8.48	176.01 uM
446165	MBS	-9.34	-0.99	2.98	-1.95	-11.36	142.89 nM

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

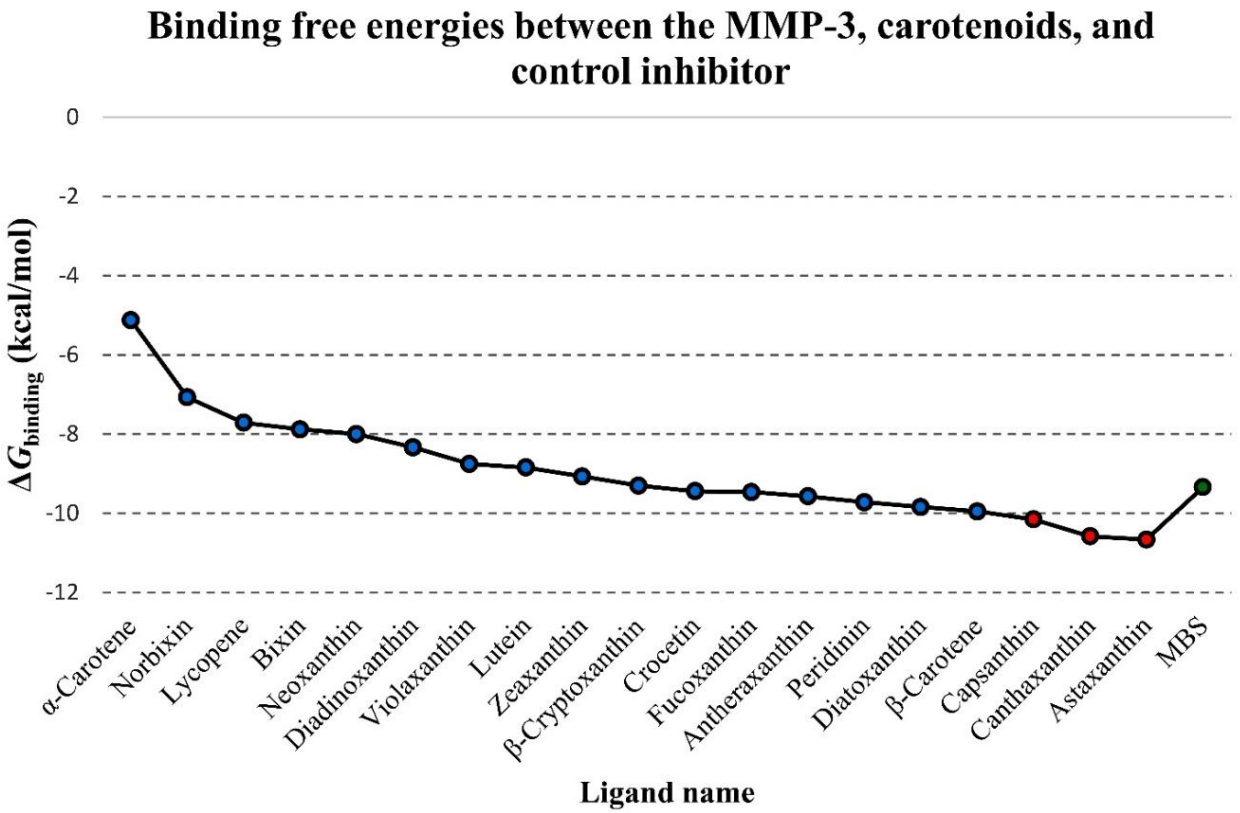


Figure 2. Comparative Analysis of the Computed $\Delta G_{binding}$ Energies (kcal/mol) for a Series of Carotenoids and the Control Inhibitor MBS Upon Interaction with the Matrix Metalloproteinase-3 (MMP-3) Catalytic Cleft
Carotenoids are ranked by binding affinity, with MBS shown with a green spot.

Table 2. Details of Energies and Ki Values Between the Top-Ranked Compounds in This Study, MBS, and the MMP-3 Active Site, in the Presence of Zinc Ions

Name	Gibbs Free Binding Energy (kcal/mol)	Unbound System's Energy (kcal/mol)	Torsional Free Energy (kcal/mol)	Final Total Internal Energy (kcal/mol)	Final Intermolecular Energy (kcal/mol)	Ki
Astaxanthin	-11.44	-1.63	+6.26	-4.85	-14.48	4.12 nM
Canthaxanthin	-10.73	-1.59	+5.67	-3.80	-14.19	13.67 nM
Capsanthin	-10.04	-1.33	+6.56	-3.06	-14.88	43.67 nM
MBS (Ctrl+)	-9.83	-0.99	2.98	-1.32	-12.48	61.91 nM

Abbreviations: MMP-3, matrix metalloproteinase-3; Ctrl, positive control; Ki, inhibition constant.

Interaction Modes

Analysis of binding interactions without the zinc ions revealed that capsanthin formed the most extensive hydrophobic network among the carotenoids, engaging six residues: Tyr655, His666, His701, His711, and Pro721. Regarding hydrogen bonding, canthaxanthin formed two H-bonds with Tyr723 and Leu722, whereas astaxanthin and capsanthin each established a single hydrogen bond with Leu664 and Tyr655, respectively. The control compound, MBS, formed one hydrogen bond with His711, supported by a broad hydrophobic profile comprising five interactions with Ala665, Val698, Tyr723, Leu718, and Leu697.

Analysis of binding interactions with the zinc ion included in the active site showed only modest changes relative to the zinc-free model. Astaxanthin no longer formed hydrogen bonds but maintained a compact hydrophobic network involving Leu664, His711, Ala669, Tyr655, and His705. Canthaxanthin retained a single hydrogen bond with Tyr723 and engaged a broad set of hydrophobic contacts with Val663, His665, Tyr668, Tyr655, His705, and Leu664. Capsanthin, which previously

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]
[DOI: 10.53208/ajpr.6.1.59]

showed the most extensive hydrophobic network among the carotenoids, lost its hydrogen bond and instead formed hydrophobic interactions with Val663, Ala665, Leu664, Tyr723, His666, Tyr668, and Tyr655. The control compound, MBS, exhibited an enhanced hydrogen-bonding pattern, forming multiple H-bonds with Leu664, Ala665, and Ala667, supported by an expanded hydrophobic interface comprising Leu718, Tyr723, Val698, His701, Leu664, and His705.

Table 3 presents a comprehensive account of all binding distances. For this analysis, interactions beyond 5.0 Å were not considered significant. Figure 3 shows the two- and three-dimensional views of the top-ranked carotenoid, MBS, within the 1HY7 active site.

Table 3. Interaction Modes Between the Top-Ranked Carotenoid, MBS, and MMP-3 Active-Site Residues

Name	Hydrogen Bond (Distance Å)	Hydrophobic Interaction (Distance Å)
A) Without the Zinc Ion		
Astaxanthin	Leu664 (3.25)	Pro721 (4.53); His701 (3.57); Val698 (3.68); Tyr723 (4.09); His666 (4.78)
Canthaxanthin	Tyr723 (4.61); Leu722 (4.19)	His666 (3.96, 4.47); Ala665 (3.31); Pro721 (4.01, 4.99)
Capsanthin	Tyr655 (4.79)	His701 (4.24); Pro721 (4.25); His711 (4.58); Tyr655 (4.49); His666 (4.77, 4.99)
MBS (Ctrl+)	His711 (4.38)	Ala665 (4.03); Val698 (4.38); Tyr723 (4.84); Leu718 (4.63); Leu697 (4.41)
B) Including the zinc ion		
Astaxanthin	Na	Leu664 (4.19); His711 (4.31); Ala669 (4.43); Tyr655 (4.88); His705 (3.55)
Canthaxanthin	Tyr723 (4.63)	Val663 (4.95); His665 (4.06); Tyr668 (3.86, 4.28, 4.74); Tyr655 (3.78, 4.47); His705 (3.94); Leu664 (4.43)
Capsanthin	Na	Val663 (3.86); Ala665 (3.43); Leu664 (4.13); Tyr723 (4.58); His666 (4.17); Tyr668 (3.81); Tyr655 (4.92)
MBS (Ctrl+)	Leu664 (3.64, 4.2); Ala665 (3.46); Ala667 (3.81)	Leu718 (4.29); Tyr723 (4.47); Val698 (4.81); His701 (4.22); Leu664 (4.51); His705 (4.3)

Abbreviations: MMP-3, matrix metalloproteinase-3; Ctrl, Positive control; Na, not available.

Discussion

Dental caries is a multifactorial disease involving the demineralization of the tooth's inorganic structure and the subsequent degradation of its organic components, leading to cavity formation. Oral cariogenic bacteria primarily drive the etiology, with numerous exogenous and endogenous risk factors influencing its progression [30]. Ozmen et al. [31] revealed a significant association with a specific MMP-3 polymorphism (rs679620), finding a statistically significant difference ($P < 0.001$) in its frequency between a "high caries risk" cohort (defined as DMFT ≥ 14) and a "caries-free" control group (DMFT = 0).

Epidemiological and clinical evidence underscores the role of carotenoids in mitigating a spectrum of non-communicable diseases, including cancer, respiratory disorders, obesity, diabetes, cardiovascular disease, and impaired neurodevelopment [32-37]. Building on their therapeutic potential, this study computationally evaluated specific organic carotenoids as MMP-3 inhibitors.

In this study, *in silico* analysis demonstrated that among the 19 carotenoids evaluated, astaxanthin, canthaxanthin, and capsanthin exhibited the highest binding affinities for the MMP-3 catalytic domain. Notably, their predicted inhibition constants (Ki values of 15.24 nM, 17.58 nM, and 36.01 nM, respectively) were superior to those of the control inhibitor, MBS (Ki = 142.89 nM), indicating a strong theoretical potential for competitive enzyme inhibition. Also, the molecular docking models revealed that the stability of these top carotenoid–MMP-3 complexes is primarily driven by extensive hydrophobic networks, interacting with key residues such as Tyr655, His666, His701, and Pro721, rather than relying strictly on hydrogen bonding. Furthermore, including the catalytic zinc ion in the structural models did not alter the overall binding hierarchy of the top compounds, demonstrating that their affinity is robust and largely depends on effective accommodation within the enzyme's

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

hydrophobic cleft. However, these findings are based on static molecular docking predictions; therefore, subsequent in vitro enzymatic assays and *in vivo* studies remain essential to validate true therapeutic efficacy.

Astaxanthin (3,3′-dihydroxy-4,4′-diketo-β,β′-carotene), a carotenoid primarily derived from marine organisms, such as shrimp, krill, crustaceans, fish, and birds, as well as the microalga Haematococcus pluvialis, has gained significant attention for its potent antioxidant, anti-inflammatory, and antiproliferative properties [38-41].

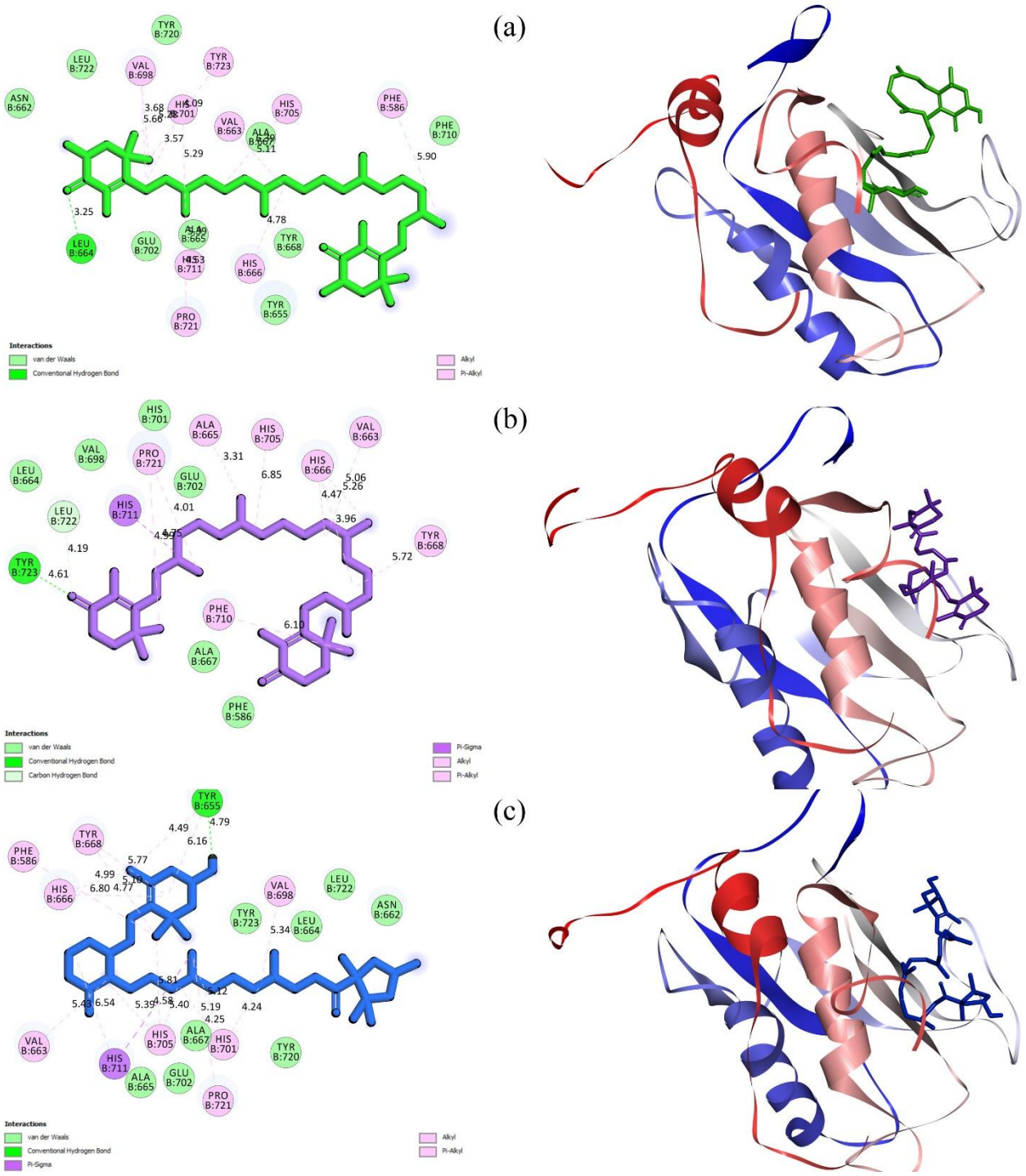


Figure 3. Binding Posing Analysis of the Most Potent Inhibitors

Two-dimensional (left) and three-dimensional (right) representations illustrate the molecular interactions and spatial occupancy of (a) Astaxanthin, (b) Canthaxanthin, (c) Capsanthin, and (d) MBS bound to the 1HY7 active site.

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

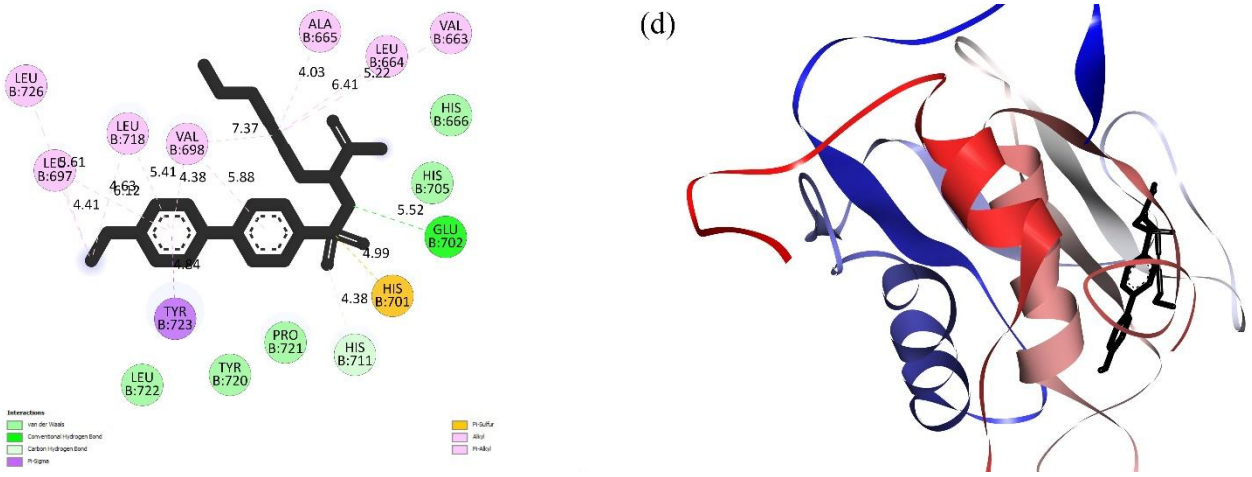


Figure 3. Continued.

The inhibitory profile of astaxanthin is further supported by Lee et al. [42], who demonstrated its ability to suppress MMP-7 and MMP-10 expression in gastric epithelial cells, consequently reducing cell invasion and migration. This cellular effect was mechanistically driven by the downregulation of the PI3K/AKT/mTOR/NF-κB signaling pathway. The strong binding affinity for MMP-3 observed in our study converges with this established biological activity, strengthening the hypothesis that astaxanthin functions as a broad-spectrum MMP inhibitor. The MMP-inhibitory effect of astaxanthin is further supported by Zhang et al. [43], who reported that its administration in a rat model of subarachnoid hemorrhage significantly suppressed MMP9 expression and activity. This inhibition was directly correlated with improved neurological function and preserved blood-brain barrier integrity. The potent inhibition constant (Ki = 15.24 nM) for MMP-3 determined in our investigation aligns with the observed *in vivo* reduced activity of a related MMP. This concordance suggests that the high-affinity binding captured in our model provides a plausible mechanistic basis for the documented therapeutic outcomes.

Canthaxanthin is an orange-red ketocarotenoid pigment found in nature and synthesized industrially. It occurs across diverse biological kingdoms, is synthesized by various microorganisms—including specific bacteria, algae, and fungi—and accumulates in numerous animal tissues, such as those of fish, crustaceans, and birds [44, 45].

The high-affinity binding of canthaxanthin ($\Delta G_{binding}$= −10.58 kcal/mol) observed in our study aligns with the functional inhibition of MMP1 documented by Wakuda et al. [46]. Their work intriguingly demonstrates that canthaxanthin downregulates MMP1 mRNA not through an antioxidant pathway, but via the direct inhibition of p38 kinase. This implies that the strong binding affinity we measured may not target an MMP directly but instead reflects a potent interaction with an upstream regulatory kinase, revealing a novel, indirect mechanism for MMP suppression. Conversely, fucoxanthin, which exhibited a more moderate binding affinity ($\Delta G_{binding}$ = −9.46 kcal/mol) in our analysis, was shown to mediate its effects predominantly through reactive oxygen species suppression. This contrast underscores the divergent mechanistic strategies, direct kinase inhibition versus antioxidant activity, employed by different carotenoids to achieve anti-MMP outcomes.

Capsanthin, the principal red carotenoid in peppers, is an increasingly promising feed additive due to its potent bioactivities. Beyond its nutritional value, it plays a vital role in enhancing animal health and reproduction, primarily through its significant anti-inflammatory and antioxidant properties. Research indicates that capsanthin exhibits superior antioxidant capacity compared to other xanthophylls. Its efficacy has been demonstrated by

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

its ability to mitigate oxidative stress and suppress the expression of key inflammatory mediators, including tumor necrosis factor-α and interleukins (IL-2, IL-4, IL-6), in rat models [47-50].

The potential of carotenoids to inhibit MMPs is further supported by research on capsanthin. The current *in silico* analysis positions this compound as a high-affinity binder (ΔGb_{inding}= −10.15 kcal/mol) to the MMP active site, a finding that is substantiated by independent *in vivo* evidence. Specifically, Shanmugham et al. [50] reported that oral administration of capsanthin markedly inhibited MMP-2 and MMP-9 in a rat model of dry eye disease. The therapeutic action was shown to operate through a dual mechanism, mitigating oxidative stress while concurrently downregulating pro-inflammatory cytokines. The concordance between our predicted strong molecular interaction and the empirical inhibition of specific MMP isotypes not only reinforces capsanthin's therapeutic profile but also illustrates a consistent pattern among potent carotenoids: the convergence of direct enzyme inhibition with broader antioxidant and anti-inflammatory pathways.

This computational study has inherent limitations that warrant consideration. The binding affinities and inhibition constants are theoretical predictions from static molecular docking, a method that cannot fully replicate the dynamic behavior of biological systems. Furthermore, while the results indicate strong inhibitory potential, this premise requires experimental validation, such as *in vitro* enzymatic assays and cellular models, to confirm functional MMP inhibition.

Conclusion

In conclusion, this in silico study identified astaxanthin, canthaxanthin, and capsanthin as the most promising carotenoid candidates for targeting the MMP-3 catalytic domain. These compounds showed favorable binding energies and superior predicted inhibition constants relative to the reference inhibitor. Extensive hydrophobic contacts within the active site, particularly for capsanthin, and specific hydrogen bonds, as observed with canthaxanthin, govern binding stability. Although these computational findings provide a strong mechanistic rationale, the static nature of molecular docking necessitates future in vitro and in vivo experimental validation. If experimental studies confirm potent inhibitory activity, these naturally occurring carotenoids could serve as valuable lead compounds for developing novel, natural preventive strategies against dentin matrix degradation and dental caries.

Conflict of Interest

The authors declared no conflicts of interest.

Authors' Contributions

Study conception and design: AT and FM;

Data collection: AT and FKh;

Methodology: AT and FKh;

Interpretation of results: AT and FM;

Writing - review and editing: AT;

Supervision: AT and FM.

The manuscript was edited using GenAI for writing style, then underwent grammatical editing using Grammarly Premium software. All authors read and approved the final version of the manuscript.

Acknowledgment and Funding

The authors thank the Research Center for Molecular Medicine and the Dental Research Center at Hamadan University of Medical Sciences, Hamadan, Iran, for their support. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Informed Consent

Not applicable.

Ethical Approval

The Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran approved this study (ethics no. IR.UMSHA.REC.1403.879).

References

1. Kassebaum NJ, Smith AGC, Bernabé E, Fleming TD, Reynolds AE, Vos T, et al. Global, regional, and national prevalence, incidence, and disability-adjusted life years for oral conditions for 195 countries, 1990-2015: a systematic analysis for the global burden of diseases, injuries, and risk factors.

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

J Dent Res. 2017;96(4):380-7. [DOI: 10.1177/0022034517693566] [PMID] [PMCID]

2. Kopycka-Kedzierawski DT, Fontana M, Marazita ML, McLaren SW, Morou-Bermúdez E, O'Connor TG, et al. Dental caries: the way forward. JDR Clin Trans Res. 2025;10(1):4-6. [DOI: 10.1177/23800844241271647] [PMID] [PMCID]
3. Organization WH. Global oral health status report: towards universal health coverage for oral health by 2030: World Health Organization; 2022. [Link]
4. Jones B, Lambach M, Chen T, Michou S, Kilpatrick N, Curtis N, et al. Dental caries detection in children using intraoral scans and deep learning. J Dent. 2025;160:105906. [DOI: 10.1016/j.jdent.2025.105906] [PMID]
5. Peres MA, Macpherson LMD, Weyant RJ, Daly B, Venturelli R, Mathur MR, et al. Oral diseases: a global public health challenge. Lancet (London, England). 2019;394(10194):249-60. [DOI: 10.1016/s0140-6736(19)31146-8] [PMID]
6. James Y, Nadeem A, Carpenter F. Role of the early detection and prevention of dental caries in children: a systematic review of clinical outcomes. Cureus. 2025;17(6):e85185. [DOI: 10.7759/cureus.85185] [PMID] [PMCID]
7. Klein MI, Hwang G, Santos PH, Campanella OH, Koo H. Streptococcus mutans-derived extracellular matrix in cariogenic oral biofilms. Front Cell Infect Microbiol. 2015;5:10. [DOI: 10.3389/fcimb.2015.00010] [PMID] [PMCID]
8. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, et al. Dental caries. Nat Rev Dis primers. 2017;3:17030. [DOI: 10.1038/nrdp.2017.30] [PMID]
9. Vijayakumar A, Sarveswari HB, Vasudevan S, Shanmugam K, Solomon AP, Neelakantan P. Baicalein Inhibits Streptococcus mutans Biofilms and Dental Caries-Related Virulence Phenotypes. Antibiotics (Basel, Switzerland). 2021;10(2)215. [DOI: 10.3390/antibiotics10020215] [PMID] [PMCID]
10. Lewis NV, Aggarwal S, Borse NN, Sonawane S, Dhatavkar P, Digholkar R, et al. The effect of matrix metalloproteinase inhibitors on the microtensile bond strength of dentin bonding agents in caries affected dentin: a systematic review. J Int Soc Prev Community Dent. 2023;13(3):173-84. [DOI: 10.4103/jispcd.jispcd_5_23] [PMID] [PMCID]
11. Boukpessi T, Menashi S, Camoin L, Tencate JM, Goldberg M, Chaussain-Miller C. The effect of stromelysin-1 (MMP-3) on non-collagenous extracellular matrix proteins of demineralized dentin and the adhesive properties of restorative resins. Biomaterials. 2008;29(33):4367-73. [DOI: 10.1016/j.biomaterials.2008.07.035] [PMID]
12. Den Besten PK, Punzi JS, Li W. Purification and sequencing of a 21 kDa and 25 kDa bovine enamel metalloproteinase. Eur J Oral Sci. 1998;106 Suppl 1:345-9. [DOI: 10.1111/j.1600-0722.1998.tb02196.x] [PMID]
13. Hall R, Septier D, Embery G, Goldberg M. Stromelysin-1 (MMP-3) in forming enamel and predentine in rat incisor-coordinated distribution with proteoglycans suggests a functional role. Histochem J. 1999;31(12):761-70. [DOI: 10.1023/a:1003945902473] [PMID]
14. Salman M, Asgartooran B, Taherkhani A. Targeting matrix metalloproteinase-3 for dental caries prevention using herbal isolates: mmp3 inhibition by cinnamic acids. Int J Dent. 2024;2024:9970824. [DOI: 10.1155/2024/9970824] [PMID] [PMCID]
15. Bartali B, Semba RD. Carotenoids and healthy aging: the fascination continues. Am J Clin Nutr. 2021;113(2):259-60. [DOI: 10.1093/ajcn/nqaa364] [PMID] [PMCID]
16. Meléndez-Martínez AJ, Mandić AI, Bantis F, Böhm V, Borge GIA, Brnčić M, et al. A comprehensive review on carotenoids in foods and feeds: status quo, applications, patents, and research needs. Crit Rev Food Sci Nutr. 2022;62(8):1999-2049. [DOI: 10.1080/10408398.2020.1867959] [PMID]
17. Mapelli-Brahm P, Gómez-Villegas P, Gonda ML, León-Vaz A, León R, Mildenberger J, et al. Microalgae, seaweeds and aquatic bacteria, archaea, and yeasts: sources of carotenoids with potential antioxidant and anti-inflammatory health-promoting actions in the sustainability era. Mar Drugs. 2023;21(6). [DOI: 10.3390/md21060340] [PMID] [PMCID]
18. Sandmann G. Carotenoids and their biosynthesis in fungi. Molecules (Basel, Switzerland). 2022;27(4):1431. [DOI: 10.3390/molecules27041431] [PMID] [PMCID]
19. Di Carlo E, Augustin AJ. Prevention of the Onset of Age-Related Macular Degeneration. J Clin Med. 2021;10(15):3297. [DOI: 10.3390/jcm10153297] [PMID] [PMCID]
20. Shanaida M, Mykhailenko O, Lysiuk R, Hudz N, Balwierz R, Shulhai A, et al. Carotenoids for antiaging: nutraceutical, pharmaceutical, and cosmeceutical applications. Pharmaceuticals (Basel, Switzerland). 2025;18(3):403. [DOI: 10.3390/ph18030403] [PMID] [PMCID]
21. Sajjad W, Abbasi SW, Ali L. Molecular docking study of astaxanthin derived from radio-resistant bacterium Deinococcus sp. strain WMA-LM9 to Matrix Metalloproteinase-1, 3 (MMP-1, MMP-3). Life Sci. 2021;2(1):6-. [DOI: 10.37185/LnS.1.1.105]
22. Velázquez-Libera JL, Durán-Verdugo F, Valdés-Jiménez A, Núñez-Vivanco G, Caballero J. LigRMSD: A web server for automatic structure matching and RMSD calculations among identical and similar compounds in protein-ligand docking. Bioinformatics (Oxford, England). 2020;36(9):2912-4. [DOI: 10.1093/bioinformatics/btaa018] [PMID]

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

23. Burley SK, Berman HM, Christie C, Duarte JM, Feng Z, Westbrook J, et al. RCSB Protein Data Bank: Sustaining a living digital data resource that enables breakthroughs in scientific research and biomedical education. Protein Sci. 2018;27(1):316-30. [DOI: 10.1002/pro.3331] [PMID] [PMCID]
24. Natchus MG, Bookland RG, Laufersweiler MJ, Pikul S, Almstead NG, De B, et al. Development of new carboxylic acid-based MMP inhibitors derived from functionalized propargylglycines. J Med Chem. 2001;44(7):1060-71. [DOI: 10.1021/jm000477l] [PMID]
25. Johansson MU, Zoete V, Michielin O, Guex N. Defining and searching for structural motifs using DeepView/Swiss-PdbViewer. BMC Bioinformatics. 2012;13:173. [DOI: 10.1186/1471-2105-13-173] [PMID] [PMCID]
26. Froimowitz M. HyperChem: a software package for computational chemistry and molecular modeling. BioTechniques. 1993;14(6):1010-3. [PMID]
27. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61. [DOI: 10.1002/jcc.21334] [PMID] [PMCID]
28. Jain AN. Bias, reporting, and sharing: computational evaluations of docking methods. J Comput Aided Mol Des. 2008;22(3-4):201-12. [DOI: 10.1007/s10822-007-9151-x] [PMID]
29. de Magalhaes CS, Barbosa HJ, Dardenne LE, editors. Selection-insertion schemes in genetic algorithms for the flexible ligand docking problem. Genetic and Evolutionary Computation Conference; 2004: Springer. [Link]
30. Karayasheva D, Glushkova M, Boteva E, Mitev V, Kadiyska T. Association study for the role of matrix metalloproteinases 2 and 3 gene polymorphisms in dental caries susceptibility. Arch Oral Biol. 2016;68:9-12. [DOI: 10.1016/j.archoralbio.2016.03.007] [PMID]
31. Özmen S, Atalı PY, Ağralı ÖB, Aslan BT, Yılmaz ÖÖ, Polat T, Ulucan K. The effects of mmp3 (rs679620) and VDR (rs731236) gene polymorphisms on dental caries: a pilot study. Eur J Dent. 2025;19(02):330-6. [DOI: 10.1055/s-0044-1787978] [PMID] [PMCID]
32. Ahmed S, Hasan MM, Heydari M, Rauf A, Bawazeer S, Abu-Izneid T, et al. Therapeutic potentials of crocin in medication of neurological disorders. Food Chem Toxicol. 2020;145:111739. [DOI: 10.1016/j.fct.2020.111739] [PMID]
33. Bakac ER, Percin E, Gunes-Bayir A, Dadak A. A narrative review: the effect and importance of carotenoids on aging and aging-related diseases. Int J Mol Sci. 2023;24(20):15199. [DOI: 10.3390/ijms242015199] [PMID] [PMCID]
34. Ferreira YAM, Jamar G, Estadella D, Pisani LP. Role of carotenoids in adipose tissue through the AMPK-mediated pathway. Food Funct. 2023;14(8):3454-62. [DOI: 10.1039/d2fo03781e] [PMID]
35. Melo van Lent D, Leermakers ETM, Darweesh SKL, Moreira EM, Tielemans MJ, Muka T, et al. The effects of lutein on respiratory health across the life course: A systematic review. Clin Nutr ESPEN. 2016;13:e1-e7. [DOI: 10.1016/j.clnesp.2016.02.096] [PMID]
36. Sani A, Tajik A, Seiiedi SS, Khadem R, Tootooni H, Taherynejad M, et al. A review of the anti-diabetic potential of saffron. Nutr Metab Insights. 2022;15:11786388221095223. [DOI: 10.1177/11786388221095223] [PMID] [PMCID]
37. Sui J, Guo J, Pan D, Wang Y, Xu Y, Sun G, et al. The efficacy of dietary intake, supplementation, and blood concentrations of carotenoids in cancer prevention: insights from an umbrella meta-analysis. Foods. 2024;13(9):1321. [DOI: 10.3390/foods13091321] [PMID] [PMCID]
38. Copat C, Favara C, Tomasello MF, Sica C, Grasso A, Dominguez HG, et al. Astaxanthin in cancer therapy and prevention (Review). Biomed Rep. 2025;22(4):66. [DOI: 10.3892/br.2025.1944] [PMID] [PMCID]
39. Ma XK, Shao X, Xu T, Yan H, Teng H, Chen ZX, et al. New horizons of astaxanthin-loaded akkermansia muciniphila as an integrated dietary supplement: physicochemical structures and gastrointestinal fate. Probiotics Antimicrob Proteins. 2026;18(1):892-906. [DOI: 10.1007/s12602-025-10572-9] [PMID]
40. Mohd Shafie AS, Kamarudin SN, Meor Mohd Affandi MMR, Siran R. Exploring astaxanthin: a comprehensive review on its pharmacokinetics properties and neuroprotective potential. Nutr Neurosci. 2025;28(10):1197-224. [DOI: 10.1080/1028415x.2025.2499559] [PMID]
41. Ren F, Rao C, Xiang Q, Wen J, Dai Q, Li H, et al. Production methods, biological activity and potential application prospects of astaxanthin. Foods. 2025;14(12):2103. [DOI: 10.3390/foods14122103] [PMID] [PMCID]
42. Lee J, Lim JW, Kim H. Astaxanthin inhibits matrix metalloproteinase expression by suppressing pi3k/akt/mtor activation in helicobacter pylori-infected gastric epithelial cells. Nutrients. 2022;14(16):3427. [DOI: 10.3390/nu14163427] [PMID] [PMCID]
43. Zhang XS, Zhang X, Zhang QR, Wu Q, Li W, Jiang TW, et al. Astaxanthin reduces matrix metalloproteinase-9 expression and activity in the brain after experimental subarachnoid hemorrhage in rats. Brain Res. 2015;1624:113-24. [DOI: 10.1016/j.brainres.2015.07.020] [PMID]
44. Gaur V, Bera S. Microbial canthaxanthin: an orange-red keto carotenoid with potential pharmaceutical applications. BioTechnologia (Pozn). 2023;104(3):315-28. [DOI: 10.5114/bta.2023.130733] [PMID] [PMCID]

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]

45. Rebelo BA, Farrona S, Ventura MR, Abranches R. Canthaxanthin, a red-hot carotenoid: applications, synthesis, and biosynthetic evolution. Plants (Basel). 2020;9(8):1039. [DOI: 10.3390/plants9081039] [PMID] [PMCID]
46. Wakuda N, Manabe Y, Seko T, Sugawara T. Different suppressive effects of canthaxanthin and fucoxanthin on cellular responses of human dermal fibroblasts to ultraviolet A irradiation. Biosci Biotechnol Biochem. 2025;89(7):1038-44. [DOI: 10.1093/bbb/zbaf056] [PMID]
47. Csernus B, Szabó C, Knop R, Kidane RG, Ndunguru SF, Gulyás G, et al. Capsanthin supplementation modulates the immune response in broiler chickens under Escherichia coli lipopolysaccharide challenge. Arch Anim Breed. 2023;66(1):103-11. [DOI: 10.5194/aab-66-103-2023] [PMID] [PMCID]
48. Shah SNM, Tian S-L, Gong Z-H, Arisha MH. Studies on metabolism of capsanthin and its regulation under different conditions in pepper fruits (Capsicum spp.). Annu Res Rev Biol. 2014;4:1106. [Link]
49. Pérez-Gálvez A, Mínguez-Mosquera MI. Degradation of non-esterified and esterified xanthophylls by free radicals. Biochim Biophys Acta. 2002;1569(1-3):31-4. [DOI: 10.1016/s0304-4165(01)00229-x] [PMID]
50. Shanmugham V, Subban R. Capsanthin from Capsicum annum fruits exerts anti-glaucoma, antioxidant, anti-inflammatory activity, and corneal pro-inflammatory cytokine gene expression in a benzalkonium chloride-induced rat dry eye model. J Food Biochem. 2022;46(10):e14352. [DOI:10.1111/jfbc.14352] [PMID]

[Downloaded from ajpr.umsha.ac.ir on 2026-11-05]

[DOI: 10.53208/ajpr.6.1.59]