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REVIEW

Bixin Beyond Colour: Expanding Therapeutic Horizons Through the Integration of Pharmacological Potential with Modern Drug Design and Delivery Strategies

Ahmed Raza Hashmi[1], Mahendran Sekar[1,2], Ling Shing Wong[3], BR Prashantha Kumar[4], Nur Najihah Izzati Mat Rani[2], Vinoth Kumarasamy[5]

[1]School of Pharmacy, Monash University Malaysia, Subang Jaya, Selangor, Malaysia; [2]Faculty of Pharmacy and Health Sciences, Royal College of Medicine Perak, Universiti Kuala Lumpur, Ipoh, Perak, Malaysia; [3]Faculty of Health and Life Sciences, INTI International University, Nilai, Negeri Sembilan, Malaysia; [4]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India; [5]Department of Parasitology & Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Correspondence: Mahendran Sekar; Vinoth Kumarasamy, Email mahendran.sekar@monash.edu; vinoth@ukm.edu.my

Abstract: Bixin, a vibrant apocarotenoid derived from the seeds of *Bixa orellana* Linn. (commonly known as annatto), has been traditionally used as a natural colorant. However, recent advances have unveiled its diverse pharmacological and therapeutic potential, positioning it as a promising candidate in modern drug discovery and delivery systems. This review presents a comprehensive overview of bixin, encompassing its chemical structure, biosynthetic pathways, physicochemical characteristics, extraction techniques and novel nanodrug delivery systems. We critically examine its biological activities, including antioxidant, anti-inflammatory, anticancer, antimicrobial, neuroprotective, hepatoprotective, nephroprotective, and photoprotective effects, supported by mechanistic insights such as activation of the NRF2 pathway, suppression of STAT6 signaling, and modulation of oxidative stress and inflammatory mediators. Despite its wide-ranging bioactivities, the clinical application of bixin remains limited due to challenges related to its solubility, stability, and bioavailability. To address these limitations, innovative formulation strategies, including nanoparticle encapsulation, sustained-release systems, and polymer conjugates have been explored to enhance its pharmacokinetic profile and therapeutic efficacy. Majorly, field of nano-pharmaceutical technologies provides deep insights to overcome physiochemical challenges and enhance the therapeutic potential of bioactive agents. Nowadays, lipid-based nanodrug delivery approaches contribute in an efficient manner. Additionally, its integration into functional foods, cosmeceuticals, and photoprotective applications highlights its interdisciplinary relevance. This review uniquely bridges the gap between traditional knowledge and contemporary pharmaceutical science by consolidating current findings on bixin's pharmacological roles and delivery challenges. It also proposes future directions for translating bixin into a next-generation phytopharmaceutical through advanced formulation, mechanistic validation, and clinical evaluation. Collectively, the evidence positions bixin not merely as a natural colorant, but as a versatile bioactive molecule with significant promise in drug design, development, therapy, and delivery.

Keywords: bixin, *Bixa orellana*, drug discovery, biosynthesis, therapeutic potential, drug delivery

Introduction

Bixin is a naturally occurring apocarotenoid pigment primarily derived from the seeds of *Bixa orellana* Linn. (*B. orellana*), a plant commonly known as annatto or achiote. Native to tropical regions of the America, India, and East Africa, *B. orellana* has been traditionally used for various medicinal, cosmetic, and culinary purposes. Among the major active constituents of its seeds, bixin stands out as the primary pigment, responsible for the characteristic reddish-orange hue widely used as a natural colorant in food, cosmetics, and pharmaceuticals.[1–3] The seeds of *B. orellana* contain two major carotenoid compounds: bixin, which is oil-soluble, and its water-soluble counterpart norbixin, which results

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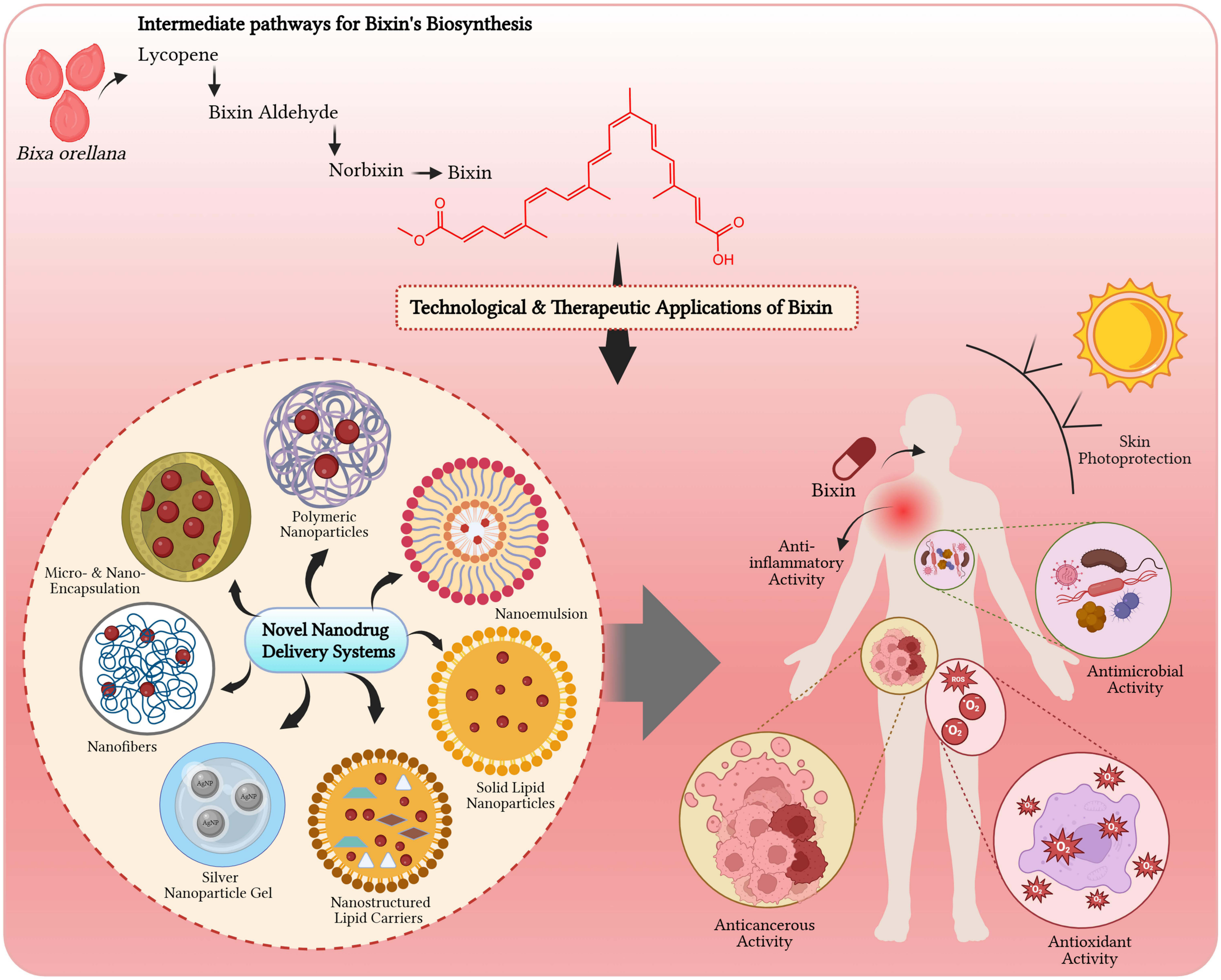
Graphical Abstract



from the alkaline hydrolysis of bixin.[3] Together, these pigments are collectively known as annatto and are utilized extensively in the food industry under the designation E160b as natural food colorants. Beyond their coloring capacity, emerging research has brought attention to the pharmacological significance of bixin and its derivatives.

Bixin exhibits strong antioxidant properties due to its conjugated double-bond structure, which facilitates free radical scavenging activity. This antioxidant potential contributes to its proposed roles in the prevention of oxidative stress-related disorders such as cardiovascular diseases and metabolic syndrome.[2,3] Furthermore, Ferreira et al (2013) demonstrated that ethanolic extracts of *B. orellana* seeds exhibit lipid-lowering effects in experimental hyperlipidemic models, supporting its traditional use in managing dyslipidemia.[4] Ethnopharmacological data also indicate that *B. orellana* has been used in folk medicine for the treatment of a wide range of conditions, including skin infections, burns, gastric ulcers, diabetes, and hypertension.[5,6] Additionally, the seeds have been found to possess antimicrobial, anti-inflammatory, and wound healing properties.[1,3] The Natural Standard Research Collaboration conducted a systematic review that supported the safety and therapeutic potential of annatto, particularly highlighting bixin's antioxidative and antimicrobial roles.[1] Apart from its health-related benefits, bixin also demonstrates insecticidal and mosquito repellent properties. Studies by de Arias and colleagues revealed that extracts of *B. orellana* deter feeding and exhibit toxic effects on insect vectors such as *Lutzomyia longipalpis*, which are responsible for the transmission of leishmaniasis.[7] Similarly, Giorgi et al[8] confirmed the mosquito repellent activity of the plant, further strengthening its use in ethnobotanical pest management practices.[8]

In plant biology, bixin has garnered interest due to its unique biosynthetic pathway as a carotenoid derivative. Rivera-Madrid et al provided an in-depth analysis of the biosynthesis of carotenoid derivatives in *Bixa orellana*, suggesting promising applications of bixin not only as a bioactive compound but also in the context of metabolic engineering for industrial pigment production.[3] Extensive ethnopharmacological records demonstrate that *Bixa orellana* has long been used in folk medicine for treating infections, inflammation, metabolic syndromes, and skin disorders.[5,6] Scientific studies have validated many of these traditional uses, highlighting the bioactivity of bixin, which includes anti-inflammatory,[9] antioxidant,[10,11] anticancer,[12] photoprotective,[10] and antimicrobial properties.[13] Recent pharmacological investigations have demonstrated that bixin can modulate multiple signaling pathways such as nuclear factor erythroid 2–related factor 2 (NRF2) pathway, adenosine monophosphate-activated protein kinase (AMPK), and suppression of signal transducer and activator of transcription 6 (STAT6) signalling (Figure 1), thereby offering therapeutic promise in oxidative stress-related diseases, cancer, kidney fibrosis, neurodegeneration, and even autoimmune diseases like multiple sclerosis.[10–12] For instance, systemic administration of bixin has been shown to mitigate solar UV-induced skin damage through NRF2 activation, while its combination with dacarbazine sensitized melanoma cells to chemotherapy via ROS-mediated apoptosis.[10,12]

Despite these promising bioactivities, bixin's therapeutic translation is hindered by major challenges in drug delivery, particularly its poor aqueous solubility, photoinstability, rapid degradation, and limited bioavailability when administered orally or topically.[3,14] Bixin, being highly lipophilic and unstable under oxidative conditions, requires innovative formulation approaches to harness its full pharmacological potential. Encapsulation techniques, including the formation of inclusion complexes with β-cyclodextrin,[15] nanoformulations,[16] and intercalation with

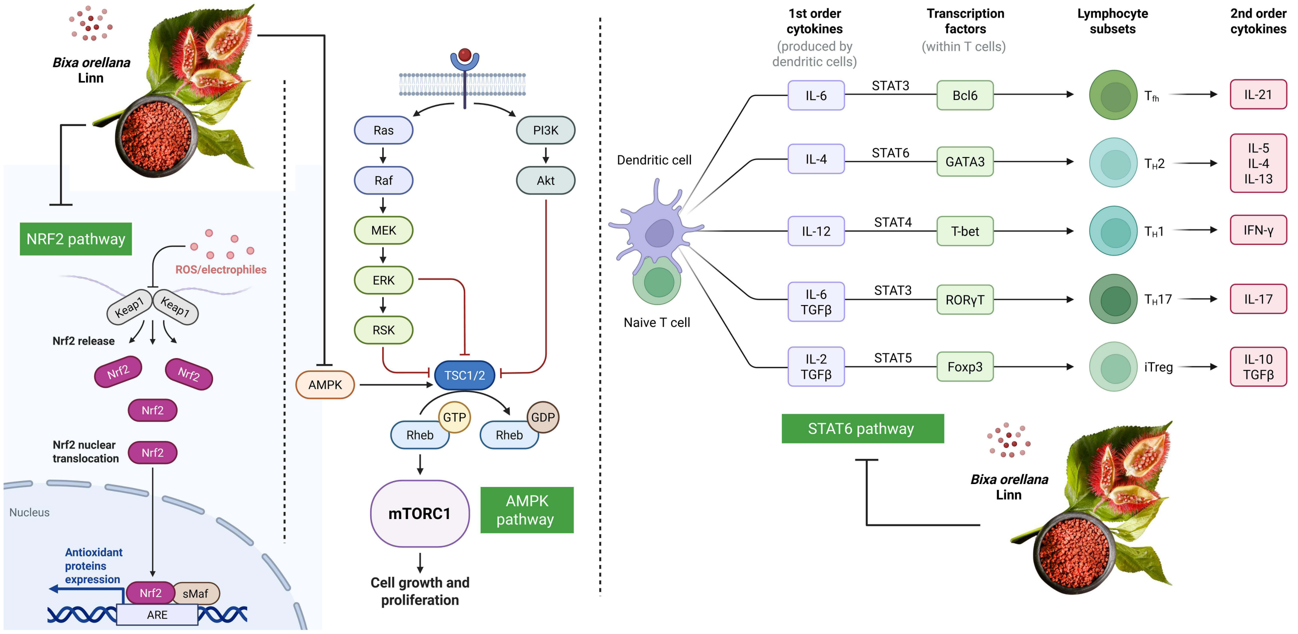


Figure 1 Proposed immunomodulatory and antioxidant mechanisms of *Bixa orellana* Linn. *Bixa orellana* Linn. exhibits antioxidant and immunomodulatory activity through several proposed molecular mechanisms. Current evidence indicates that its bioactive constituents can activate the NRF2 signalling pathway, contributing to enhanced cellular antioxidant defenses. *B. orellana* has also been reported to modulate immune responses, including effects on STAT6-associated signalling, which may influence cytokine-mediated regulation of immune cell function.

Abbreviations: AMPK, AMP-activated protein kinase; ARE, Antioxidant response element; Bcl6, B-cell lymphoma 6 protein; ERK, Extracellular signal-regulated kinase; Foxp3, Forkhead box P3; GDP, Guanosine diphosphate; GTP, Guanosine triphosphate; iTreg, Induced regulatory T cell; MEK, Mitogen-activated protein kinase kinase; mTORC1, Mechanistic target of rapamycin complex 1; NRF2, Nuclear factor erythroid 2-related factor 2; PI3K, Phosphoinositide 3-kinase; Ras/Raf, Rat sarcoma/rapidly accelerated fibrosarcoma kinases; ROS, Reactive oxygen species; RSK, Ribosomal S6 kinase; STAT, Signal transducer and activator of transcription; T-bet, T-box expressed in T cells; Tfh, T follicular helper cell; Th1, T helper type 1 cell; Th2, T helper type 2 cell; Th17, T helper type 17 cell; TGFβ, Transforming growth factor beta; TSC1/2, Tuberous sclerosis complex 1/2.

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biocompatible layered double hydroxides,[17] have been explored to enhance its stability, solubility, and targeted delivery. Moreover, conjugation with natural or synthetic polymers such as ethyl cellulose has shown potential for developing sustained-release systems.[18] In this modern era, lipid-based nanocarriers subsidize very promisingly to conquer physiochemical challenges. These strategies not only improve the pharmacokinetic profile of bixin but also expand its applicability in controlled drug delivery systems, food-grade packaging films,[19] and cosmeceuticals. Nevertheless, the full potential of bixin as a drug candidate remains underexplored, primarily due to insufficient in vivo pharmacokinetic studies, lack of standardized formulations, and limited clinical validation.

Furthermore, recent studies have shown that bixin exhibits amphiphilic characteristics, enabling it to self-assemble in aqueous media and form vesicular nanostructures capable of encapsulating hydrophobic drugs, thereby enhancing its potential for drug delivery applications.[20] Overall, bixin from *B. orellana* represents a compound of substantial interest across multiple disciplines. Its multifaceted applications in health, industry, and agriculture underscore the importance of continued research into its bioactivity, safety, and potential for therapeutic development. This review offers a comprehensive and focused analysis of bixin, emphasizing its chemistry, biosynthetic pathway from lycopene, pharmacological potential, especially in oxidative stress, cancer, metabolic, and inflammatory disorders, mechanistic pathways including NRF2, AMPK, STAT6, and ROS regulation, drug delivery challenges and innovative formulation strategies, and Industrial, food, and cosmeceutical applications. The unique value of this review lies in its integrative perspective bridging natural product pharmacology, molecular mechanisms, and advanced drug delivery approaches to support the repositioning of bixin from a natural colorant to a potential therapeutic molecule. This review further identifies current research gaps, proposes formulation opportunities, and sets a roadmap for future translational and clinical research.

Isolation of Bixin from *Bixa orellana*

Bixin, the principal apocarotenoid pigment of *B. orellana* seeds, can be isolated through solvent extraction followed by purification. Typically, dried and powdered annatto seeds are subjected to extraction using organic solvents such as ethanol or chloroform, either via maceration or Soxhlet extraction. The filtrate is concentrated under reduced pressure to yield a crude reddish-orange extract. To purify bixin, the extract is partitioned with petroleum ether or hexane, allowing the lipophilic bixin to separate into the organic phase, which is then washed, evaporated, and recrystallized from ethanol if needed. Alternatively, alkaline hydrolysis using NaOH followed by acidification can convert bixin to norbixin for water-soluble applications.[3,12,14,21]

Chemistry of Bixin

Bixin (methyl hydrogen 9′-cis-6,6′-diapocarotene-6,6′-dioate_$C_{25}H_{30}O_4$)[22] is a reddish-orange crystalline powder having 25 carbons with a molecular weight of 394.5 g/mol.[23] However, its exact monoisotopic mass is 394.21 Da. Bixin, like carotenes, has nine conjugated double bonds but features carboxyl groups instead of hexene rings at both ends. There are two forms of bixin, 9′-Z-bixin and all E-bixin, as mentioned in Figure 2. Both forms are water insoluble, though hydrolysis yields norbixin, a water-soluble diacid. All three compounds are known for their bright red-orange color. The carboxylic acid and methyl ester groups are present in its chemical framework along with double bonds, which makes it lipid soluble.[9] Although this existence of a double bond confers on bixin high sensitivity to oxygen, heat and light.[22] Its melting point is consistently reported around 197°C. UV-visible analysis in chloroform typically shows strong absorbance at 472 and 503 nm, confirming its extended conjugated system.[18] Fourier transform infrared spectroscopy (FTIR) spectra reveal key functional groups, including methyl (C–H), hydroxyl (O–H), carbonyl (C=O), and ester linkages.[23] Nuclear magnetic resonance (NMR) spectroscopy studies, including both ^{1}H and ^{13}C-NMR, have identified characteristic signals corresponding to methyl esters, conjugated double bonds, and other structural features, with COSY spectra supporting proton connectivity along the polyene chain.[21] Mass spectrometric analysis further confirms the molecular ion (m/z 395) and fragmentation patterns typical of its structure, while GC-MS highlights fragment ions consistent with carboxylic acid and conjugated chain breakdown.[12,24]

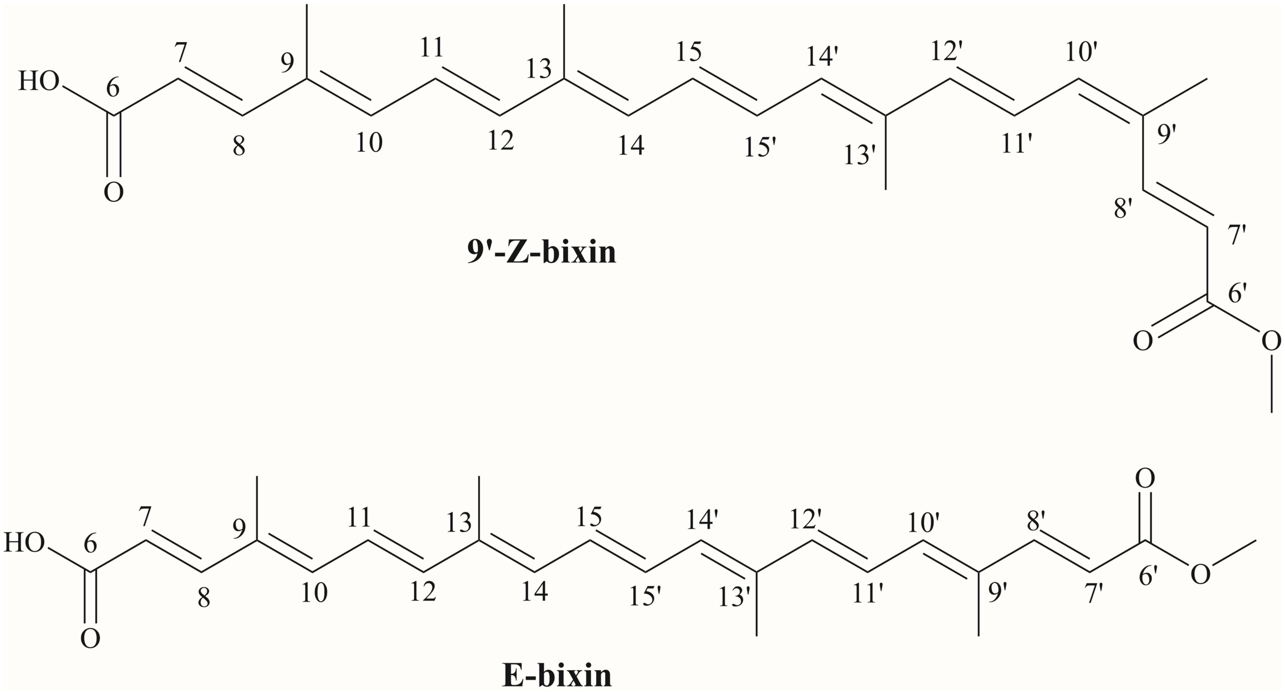

Figure 2 Chemical Structures of Bixin.

Biosynthesis of Bixin

Carotenoids, including bixin, are terpenoids synthesized from the C5 precursor isopentenyl diphosphate, which is converted to geranylgeranyl pyrophosphate (GGPP). In bixin biosynthesis, two GGPP molecules condense to form phytoene, via an intermediate, farnesylfarnesyl pyrophosphate (FFPP).[25–27] Phytoene is then converted to lycopene, which undergoes oxidative cleavage by dioxygenases to form aldehyde groups. These are further oxidized to carboxylic acids by aldehyde dehydrogenase and one is methylated by norbixin carboxyl methyltransferase to form bixin. A cis-to-trans isomerization also occurs during this process. Although plants encode many methyltransferases, only a few are involved in carboxyl group methylation, and in *Bixa*, this specific enzyme completes the synthesis of bixin (Figure 3).[24,28]

Therapeutic Potential of Bixin

Because of diverse pharmacological properties, bixin has attracted growing attention for pharmaceutical utilization. Reported literature underscores the broad therapeutic potential of this molecule, which warrants further pharmacological and clinical investigation.[29]

Antioxidant Activity

Bixin exhibits potent antioxidant properties that have garnered attention in both the health and food industries. Natural carotenoids like bixin, β-carotene, lutein, and lycopene function primarily through their antioxidant capabilities, modulating oxidative stress and reducing inflammation by suppressing pro-inflammatory mediators and enhancing cytoprotective Phase II enzymes such as NQO1 and HO-1.[30] These actions are critical in preventing oxidative stress-related disorders including, cardiovascular diseases (CVDs), type 2 diabetes (T2D), neurodegenerative conditions, and cancer. As noted by TS Tonny,[31] bixin in combination with tocotrienols, displays strong antioxidant efficacy, making it a potential agent in managing diabetes and its complications. Similarly, Assis et al[32] demonstrated that bixin-enriched yogurt significantly improved oxidative biomarkers in streptozotocin (STZ)-induced diabetic rats, elevated HDL levels, and mitigated lipid and carbohydrate disturbances. In hepatic models, bixin has been shown to reduce oxidative liver damage caused by carbon tetrachloride (CCl_4), affirming its hepatoprotective effects through ROS scavenging and

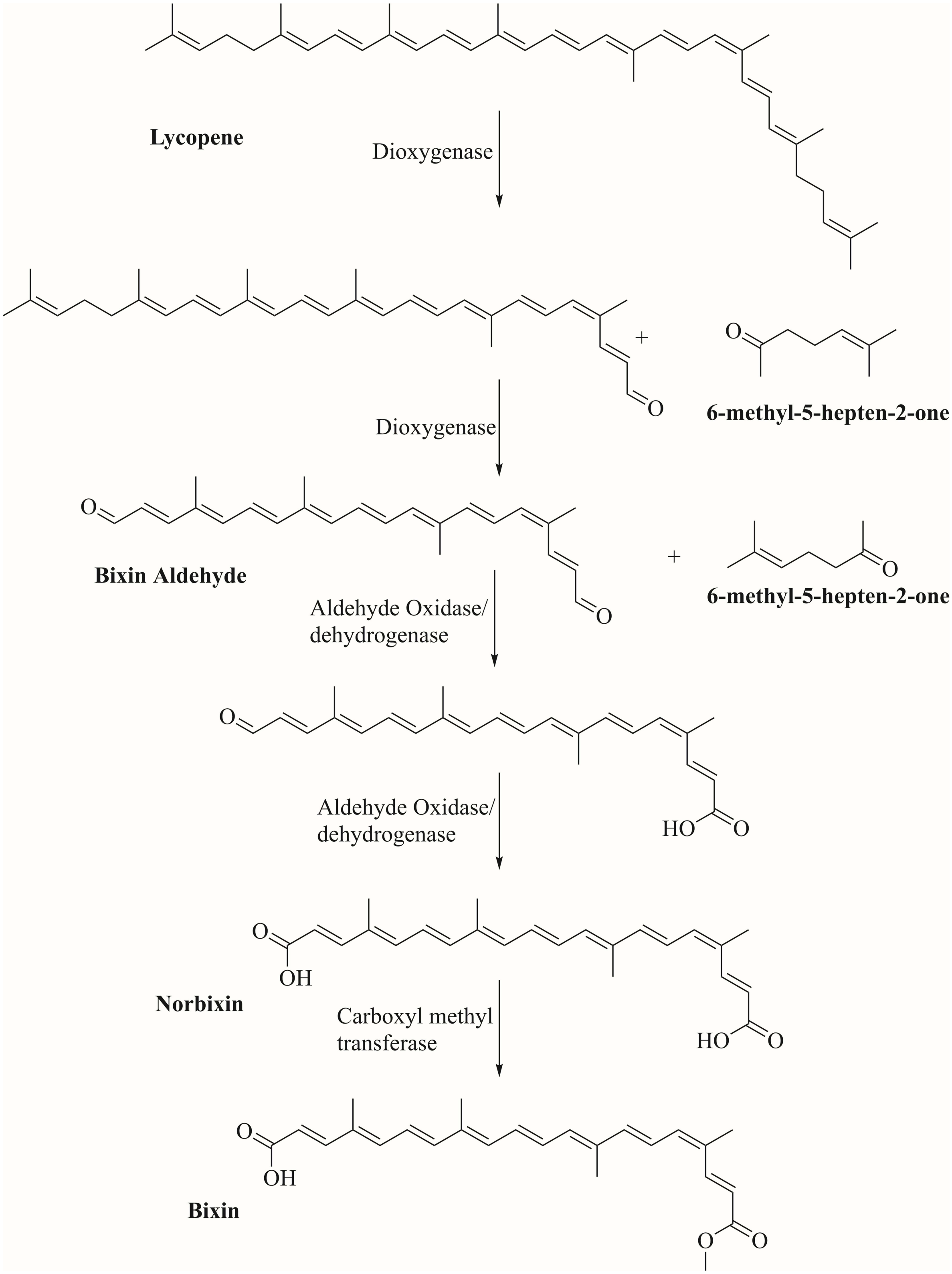

Figure 3 Biosynthesis of Bixin.

membrane stabilization.[11] Furthermore, in the context of food preservation, bixin-loaded cassava starch films developed by Pagno et al[19] were thermally stable and homogenous, effectively preventing oxidative spoilage, thereby extending shelf life. Based on the above findings, bixin activates NRF2-mediated pathways, confirming its role as a systemic antioxidant capable of protecting cells and tissues from free radical-induced damage.

Anticancer Activity

Bixin has emerged as a promising natural compound with significant anticancer potential, owing to its ability to modulate oxidative stress and intracellular signaling pathways. A recent study by Qiu et al[33] demonstrated that bixin suppresses colorectal cancer (CRC) progression by activating the AMPK/PERK/eIF2α pathway, thereby inducing endoplasmic reticulum stress and apoptosis in tumor cells, highlighting a novel preventive strategy against CRC. In lung cancer models, Nurcahyanti et al[34] explored bixin in combination with fucoxanthin and reported that this dual carotenoid therapy selectively sensitized cisplatin-resistant A549 and HeLa cancer cells, enhancing drug efficacy based on the Chou–Talalay synergy model. Supporting this, de Oliveira Júnior et al[12] reported that bixin induced reactive oxygen species (ROS)-mediated cytotoxicity in human A2058 melanoma cells carrying the BRAF^V600E mutation, which confers resistance to dacarbazine. Co-treatment with bixin and dacarbazine significantly lowered the IC_{50} values, suggesting synergistic interaction and restoration of chemosensitivity. Moreover, data from Tibodeau et al[35] revealed that cis-bixin displayed potent cytotoxicity across multiple tumor cell lines, including drug-resistant multiple myeloma cells from patients. Notably, cis-bixin selectively inhibited both thioredoxin (Trx) and thioredoxin reductase (TrxR1) key regulators of redox homeostasis leading to ROS accumulation and selective cancer cell death. These findings indicate bixin's ability to trigger redox imbalance and stress-mediated apoptotic pathways, underlining its value as a chemosensitizing and anticancer agent.

Skin Photoprotection

Bixin has emerged as a potent natural photoprotective agent, largely due to its ability to activate NRF2-mediated cytoprotective responses. Solar ultraviolet (UV) radiation is known to induce genotoxic, oxidative, and inflammatory damage, leading to skin aging and carcinogenesis.[10,36] Topical application of bixin has been shown to prevent such damage by activating the NRF2 pathway, which upregulates antioxidant defenses including glutathione synthesis and Phase II detoxifying enzymes. Rojo de la Vega et al[36] provided the first in vivo evidence using SKH1 and C57BL/6J mouse models, demonstrating that topical bixin significantly reduced UV-induced epidermal hyperproliferation, oxidative DNA damage (Figure 4), and pigmentation loss in Nrf2+/+ mice, but not in Nrf2−/− counterparts, confirming NRF2-dependency. Similarly, systemic administration of bixin attenuated skin oxidative stress and inflammation in a genotype-dependent manner, reinforcing the feasibility of dietary bixin as a systemic skin protectant.[10] Schmidlin et al[37] further confirmed that bixin pre-treatment elevated cellular glutathione levels and reduced radiation-induced dermatitis and oxidative stress in mice, thus highlighting its role in mitigating ionizing radiation toxicity through NRF2 activation. Collectively, these findings suggest that bixin may serve as an effective adjunct to conventional sunscreen, offering both topical and systemic protection against photodamage. Supporting this, Raddatz-Mota et al[38] emphasized the nutraceutical and dermatological relevance of bixin, norbixin, and tocotrienols derived from *B. orellana*, underscoring their industrial potential for skin health applications.

Antimicrobial Activity

Bixin exhibits significant antimicrobial activity, making it a promising agent in food preservation, dental applications, and therapeutic formulations. Ultrasound-assisted extraction studies have revealed that the antibacterial effects of annatto seed extracts are largely attributed to carotenoids like bixin and phenolic compounds such as catechin, chlorogenic acid, chrysin, butein, hypoaletin, and xanthoangelol.[13] These extracts demonstrated strong inhibitory activity against common foodborne pathogens like *Bacillus cereus* and *Staphylococcus aureus*, suggesting their potential use in food-safe antimicrobial coatings and packaging. Furthermore, Quiroz et al[39] emphasized the interest in annatto seed extracts for developing natural preservatives due to their antimicrobial and antioxidant synergies. In the context of dental care, Silva et al[40] investigated the potential of Urucum (*B. orellana*) extract as an adjunct to Papacarie Duo™, a chemomechanical

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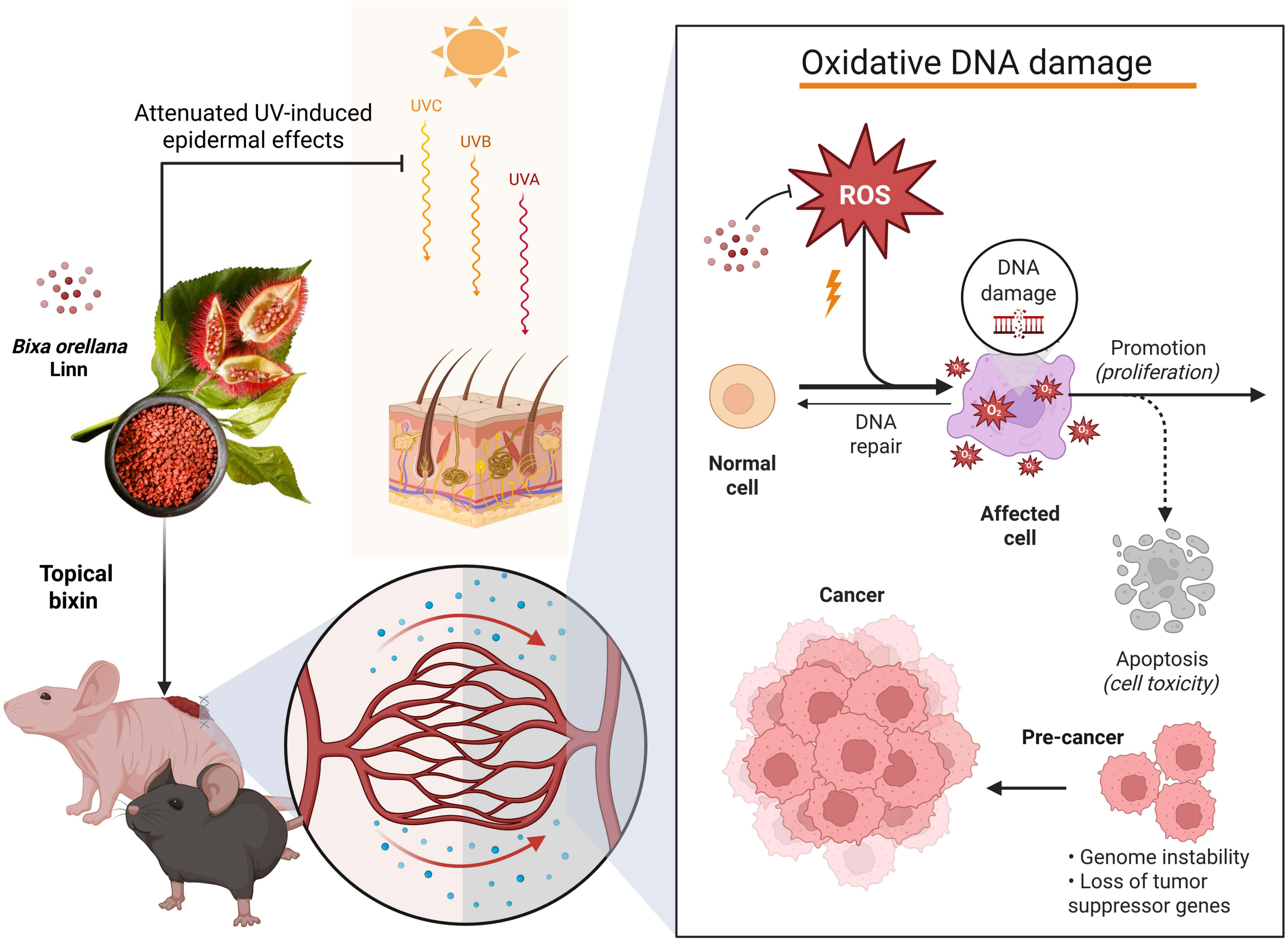

Figure 4 Protective effects of *Bixa orellana* Linn. (bixin) against UV-induced oxidative DNA damage. Topical application of bixin attenuates ultraviolet (UV)-induced epidermal damage. Exposure to UVA and UVB promotes excessive generation of reactive oxygen species (ROS), leading to oxidative DNA damage. When left unrepaired, damaged cells may undergo abnormal proliferation, genomic instability, and loss of tumor suppressor gene function, contributing to pre-cancerous changes and skin carcinogenesis. Bixin demonstrates a protective role by reducing oxidative stress, supporting DNA repair, mitigating cellular toxicity, and preventing progression toward cancerous transformation.

caries removal agent composed of papain and chloramine. The study demonstrated that cis-bixin, the primary water-soluble pigment in Urucum, did not compromise collagen structure and exhibited promising antimicrobial activity suitable for antimicrobial photodynamic therapy (aPDT) applications. These findings not only support the antimicrobial role of bixin in clinical settings but also highlight its potential for integration into functional food products, oral hygiene formulations, and biomedical coatings.

Anti-Inflammatory Activity

Bixin, a natural apocarotenoid derived from *Bixa orellana*, has demonstrated substantial anti-inflammatory potential in both acute and chronic disease models. In a recent study, nanoparticle-formulated bixin (npBX) was evaluated for its ability to prevent lung inflammation and oxidative stress induced by cigarette smoke (CS). The results showed that npBX significantly reduced leukocyte counts and TNF-α levels in the bronchoalveolar lavage fluid (BALF) of CS-exposed mice. Furthermore, npBX markedly inhibited CS-induced elevations of malondialdehyde (MDA) and protein carbonylation (PNK) in lung tissues, demonstrating its efficacy in attenuating acute lung inflammation.[16] In the context of age-related macular degeneration (AMD), a leading cause of visual impairment among the elderly norbixin, a derivative of bixin, has been shown to synergize with crocetin (from *Crocus sativus*) to slow the progression from intermediate AMD (iAMD) to late-stage AMD, including both neovascular AMD (nAMD) and geographic atrophy (GA).[41] Similarly, Xue et al[42] reported that bixin treatment significantly reduced inflammatory cell infiltration and fibrotic remodeling in the lungs, highlighting its therapeutic promise for other inflammation-associated diseases. Moreover, Roehrs et al[43] proposed that norbixin may serve as a dietary intervention to reduce postprandial inflammatory and oxidative stress responses following high-calorie meals. Together, these findings underscore bixin and its derivatives as promising natural compounds for the prevention and treatment of inflammation-related disorders.

Antinociceptive Activity

Along with anti-inflammatory activity, bixin also exhibits its novel capacity for pain reduction.[29] Pacheco et al performed a study and claimed to firstly report the antinociceptive activity of bixin without showing sedative effect.[9] The analgesic potential of bixin was evaluated through several experimental pain models in rodents. Initially, the analgesic effect of bixin was analyzed in the formalin test by consuming rats, in which bixin (at 15 or 30 mg/kg) remarkably reduced the number of flinches in both the early neurogenic and late inflammatory phases, reflecting that it can act on both immediate nociceptor stimulation and subsequent peripheral inflammatory pain. In the acetic acid-induced writhing test, bixin (at 27 or 53 mg/kg in mice) markedly decreased the number of writhing responses, likely due to its anti-inflammatory and antioxidant effects, along with its ability to reduce neutrophil migration and inhibit prostaglandin production. Moreover, in the hot plate test, the higher dose of bixin (30 mg/kg in rats) significantly prolonged the response time to a thermal stimulus, indicating involvement of central pain modulation mechanisms. Significantly, these antinociceptive features were not associated with sedation, as bixin-treated rats showed no impairment in locomotor activity in the open field test. Together, these results reflect that bixin exhibits both peripheral and central analgesic actions without affecting normal motor function.[9]

Anti-SARS-CoV-2 Activity

Bereczki et al highlighted the potential of bixin for showing remarkable antiviral activity against SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2).[44] It is the first time when apocarotenoids were listed among potential SARS-CoV-2 inhibitors. In order to analyze the anti-SARS-CoV-2 activity of bixin, the Vero E6 cell line (African green monkey kidney epithelial cells) was utilized across three orthogonal assays. The viral RNA reduction assay, cytopathic effect (CPE) reduction assay, and the immunofluorescence assay (IFA) determined an EC_{50} of 5.9 ± 1.7 µM, 14 ± 3.5 µM and 28 ± 8.8 µM, respectively. That confirmed bixin's activity in inhibiting viral replication by visualizing the reduction in SARS-CoV-2 nucleoprotein expression. Moreover, bixin was found to be both effective and safe, as it exhibited a high therapeutic index (>17) and very low toxicity, with a CC_{50} value greater than 100 µM. Further mechanistic studies demonstrated that bixin acts by blocking certain host enzymes that help the virus enter cells. In particular, it inhibited Cathepsin L and Cathepsin B with an IC_{50} of 41.92 ± 1.12 µM and 14.56 ± 1.71 µM, respectively. These conclusions suggest that bixin interferes with viral entry by targeting key proteases in the host cell.

Other Therapeutic Potential of Bixin

In a study conducted by Yu et al,[45] bixin treatment significantly reduced neuro-inflammation and demyelination in experimental autoimmune encephalomyelitis (EAE) mice, a model of multiple sclerosis (MS), primarily through ROS scavenging via activation of the NRF2 signaling pathway. These findings suggest that bixin holds promise as a potential therapeutic candidate for MS. Similarly, Li et al[46] investigated the effect of bixin on renal interstitial fibrosis, reporting that bixin administration suppressed STAT6 induction by enhancing ubiquitination (but not acetylation) of STAT6, ultimately reducing its phosphorylation and stability. This mechanistic insight supports the therapeutic role of bixin in attenuating kidney fibrosis via inhibition of the STAT6 pathway. Moreover, bixin is among several carotenoids identified with preventive potential against Alzheimer's disease (AD), a prevalent and devastating neurodegenerative disorder affecting the aging population. As discussed by Lakey-Beitia et al,[47] carotenoids like bixin may serve as innovative therapeutic agents for AD and related conditions due to their antioxidant and neuroprotective actions. In another study, Tao et al[48] identified bixin as a classical NRF2 inducer that protects against lung injury in a ventilator-induced lung injury (VILI) model. Mechanistically, bixin was found to activate NRF2 signaling in vitro by inhibiting KEAP1-C151–dependent ubiquitination and degradation of NRF2, leading to elevated NRF2 responses in vivo upon intraperitoneal (IP) injection. Notably, bixin treatment restored normal lung morphology and reduced both oxidative DNA damage and inflammatory responses in mice subjected to mechanical ventilation (MV), a common therapeutic intervention for respiratory distress. These collective findings support the development of NRF2 activators like bixin as potential pharmacological agents to prevent lung injury during MV and other oxidative stress–related conditions.

Structurally Related Compounds

Bixin is the principal apocarotenoid identified in the seeds of *B. orellana*, with numerous studies extensively reporting the presence of its derivatives and isomers. Among these, norbixin and methyl-bixin are well-documented. Additionally, structural analogues such as 6,8′-diapocarotene-6,8′-dioic acid and 6,7′-diapocarotene-6,7′-dioic acid have also been isolated from *B. orellana* extracts, as illustrated in Figure 5.[12]

In an effort to discover applications for bixin other than its predominant use as a colorant, the reaction of bixin with ethyl cellulose in acetic anhydride, giving a Michael addition product, bixinethylcellulose ether was reported, as demonstrated in Figure 5.[18] Bixin–β-cyclodextrin (β-CD) inclusion complexes prepared via co-precipitation have been reported. The complexation enhanced the intensity of bixin's color and significantly improved its water solubility. When applied in curd formulations, the bixin–β-CD complex did not alter the initial characteristics of the product and was well

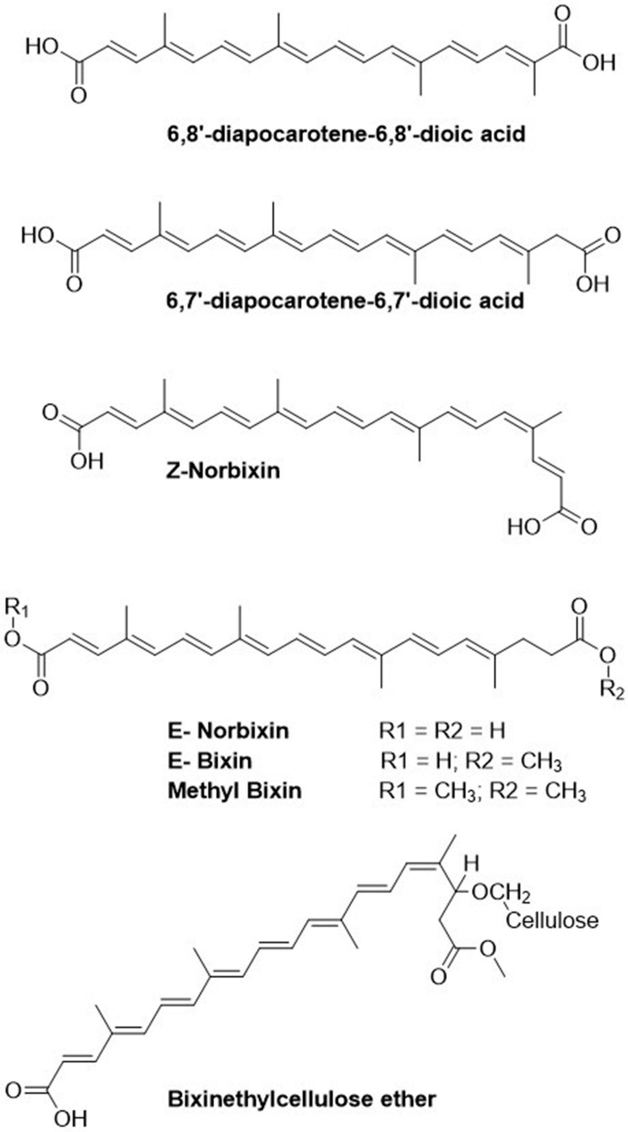

Figure 5 Structurally Related Bixin Molecules.

accepted in sensory evaluations. These findings suggest that the complexation of bixin with β-CD facilitates its incorporation into low-fat food products, thereby expanding its potential for industrial applications.[15] The synthetic conditions for intercalating norbixin into layered double hydroxides (LDHs) were systematically evaluated. LDH samples containing M^{2+}/Al^{3+} (M = Mg, Zn) were prepared using the co-precipitation method. Intercalation into the inorganic layers significantly enhanced the thermal stability of norbixin. Notably, the free radical scavenging activity of norbixin was preserved following immobilization within the LDH matrix. Given the biocompatibility of zinc- and magnesium-based LDHs, combined with the known biological activity of norbixin, these hybrid materials demonstrate strong potential for biomedical applications.[49]

Physiochemical Challenges for Bixin

Bixin is a liposoluble apocarotenoid acquired from natural sources ie, annatto seeds.[9,50] The Food and Drug Administration (FDA) endorsed it for colorant and additive purposes.[51] Furthermore, it also got permitted for therapeutic uses in pharmaceutical industries because of its multiple pharmacological responses.[45,52] In spite of that, bixin bears stability issues due to the existence of conjugated double bonds along with long chain length that lead to the development of chemical instability and degradation.[53] Moreover, in the presence of excessive heat, high pH, light and oxygen, bixin gets unstable.[54] In that, all these factors affect the solubility and decreasing the bioavailability of the bixin compound[55] and make it less suitable for therapeutic drug delivery, as illustrated in Figure 6. However, if we talk about its permeability, then bixinoids present high gastrointestinal permeability.[56] Firstly, we have to overcome the solubility

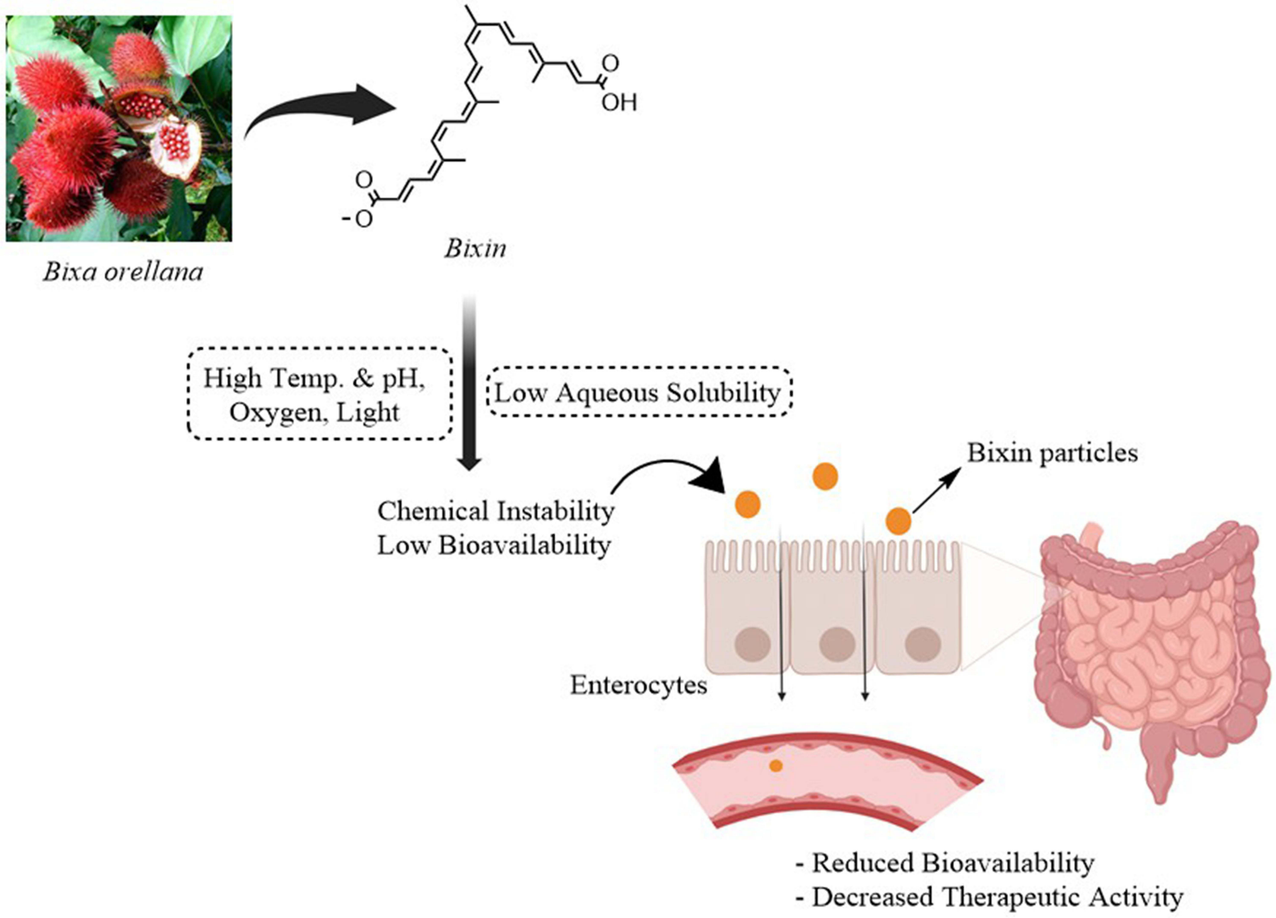

Figure 6 Bixin acquired from *Bixa orellana*, its physiochemical features ie, chemical instability, poor water solubility and low bioavailability, affects the therapeutic/pharmacological activity.

and bioavailability-related issues to potentiate the pharmacological behavior and patient compliance related to the practice of bixin.

Improving Bixin's Stability, Solubility and Bioavailability via Novel Nano-Drug Delivery Systems

The physiochemical features, like biocompatibility, reduced particle size, improved surface area and biodegradability of novel nanotechnology or nano-drug delivery carriers, open new doors for conveying the bioactive substances.[57] Nanocarriers like liposomes, niosomes, micro- and nano-emulsions, micelles, polymeric nanocarriers, nanogels, nanofibers, nanotubes, solid lipid nanoparticles and nanostructured lipid carriers,[58] along with nanoencapsulation strategies,[59,60] have contributed well and not only improve the bioavailability but also safeguard the bixin from the environment's detrimental effects. Moreover, the physiochemical properties of a given compound ie, solubility and chemical stability issues, reformed via engagement of nano-drug delivery systems.[61] Scientifically, it might be summarized that the nano-based technology is a promising approach to improve the stability, prompt the solubilization, enhance the shelf-life and improve bioactivity[30] with a controlled drug release profile of the bioactive agents.[62] These approaches have sufficient potential in designing the bixin-loaded nano-drug delivery systems with improved solubility and bioavailability behavior.[52] A diagrammatic illustration that indicating the various novel nano-drug delivery systems to improve bixin's stability and bioavailability are mentioned in Figure 7.

Nano-Drug Delivery Strategies Promoting Bixin's Bioavailability

Bixin-loaded solid lipid particles (SLNs) were designed through glycerol monostearate and trimyristin as lipid matrices and for stabilization, egg lecithin and soya were incorporated.[63] The formulation was designed via hot homogenization and characterized for in vitro drug release profile in addition to in vivo study to analyze the hepatoprotective function of a given compound by using Wistar rats. FTIR showed the compatibility of bixin with other incorporated ingredients. The drug loading and entrapment efficiency were found to be 17.96% and 99%, respectively, for bixin-loaded SLNs. The initial burst release followed by sustained release for the drug was monitored while in vitro studies were performed. In an in vivo study, bixin-loaded SLNs demonstrated a better reduction in marker enzymes in order to treat the paracetamol-induced hepatotoxicity in contrast to bixin suspension. Better appearance of therapeutic response indicates the improved drug's bioavailability.[63]

A study was conducted to develop a silver nanoparticle gel containing bixin using a green synthesis approach for topical delivery.[64] The formulation was evaluated for its stability and therapeutic efficacy, demonstrating promising results in terms of sustained activity and potential for topical application. For characterization of the designed formulation, FTIR, scanning electron microscopy (SEM), pH evaluation, drug content determination and compatibility studies were employed. The B16F10 cell line (murine melanoma cell line) was utilized for in vitro evaluation and the chemical carcinogen (7,12-dimethylbenz (a) anthracene) for in vivo evaluation in mice was utilized to evaluate the anticancer activity. Afterwards, a 60% inhibitory response of optimized formulation on B16F10 cell lines was monitored along with the suppression of oncogenesis on skin. It concluded at the end that the anticancer activity of designed formulation with bixin improved via nanocarriers.[64]

Bixin has demonstrated potent anti-inflammatory and antioxidant properties, which have been explored in the treatment of acute lung inflammation (ALI) associated with cigarette smoke exposure. Experimental findings suggest that bixin may help reduce oxidative stress and inflammatory responses, indicating its potential therapeutic role in managing ALI.[54] For this purpose, researchers developed bixin-loaded polymeric nanoparticles (npBX) via interfacial deposition technique, for oral treatment of ALI in mice that were pre-exposed to cigarette smoke. The designed formulation showed the polydispersity index, zeta potential and mean hydrodynamic diameter of 0.226 ± 0.006, − 21.69 ± 9.90 mV and 23.7 ± 0.2 nm, respectively. Moreover, drug loading of 24.23 ± 0.41 mg/g was calculated. After in vivo evaluation, it was monitored that the treatment with npBX provides support as a therapeutic weapon for treatment. Improved treatment of ALI indicated the improved bioavailability of orally administered npBX.[54]

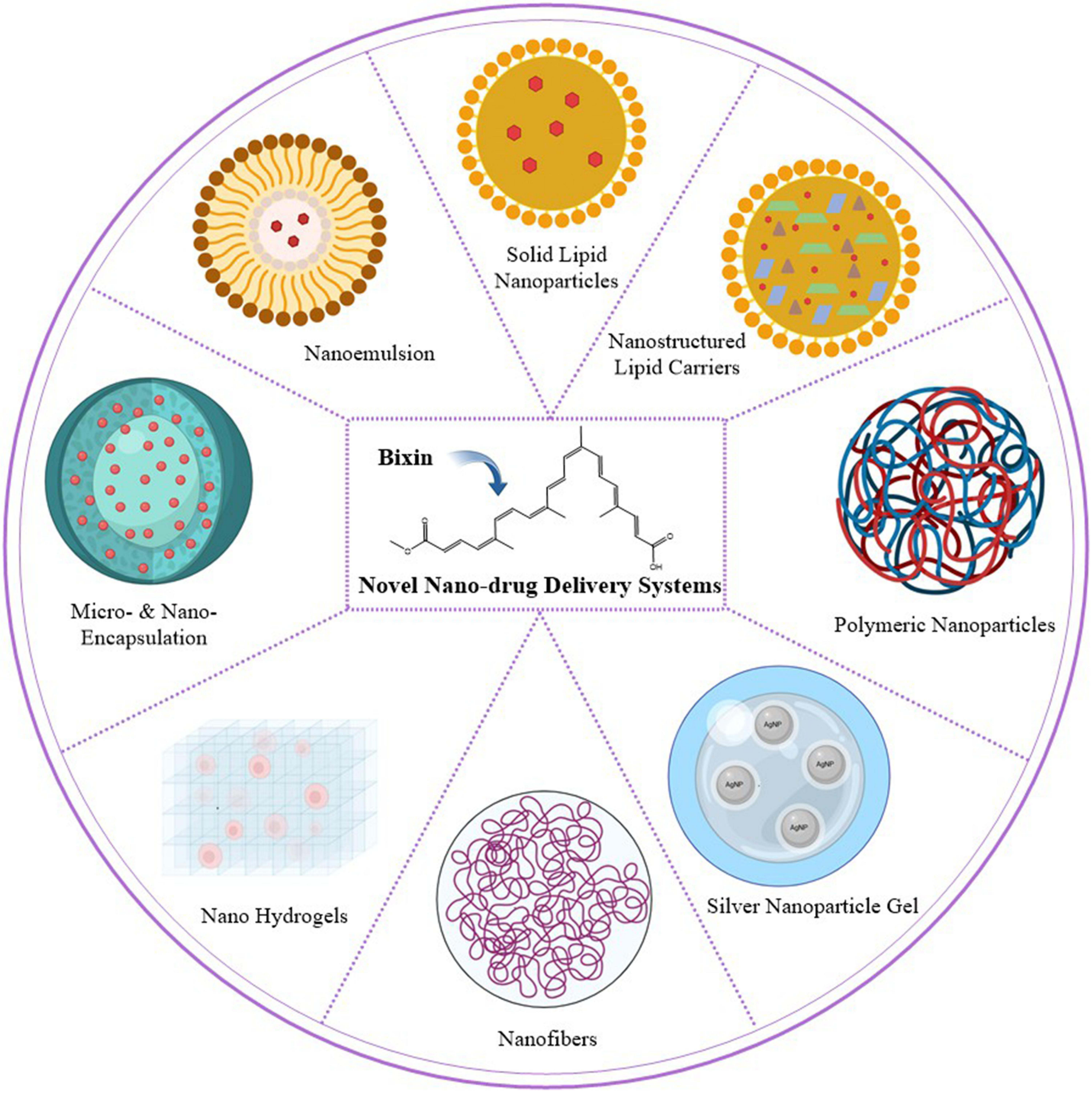

Figure 7 Various nanodrug delivery systems employing for delivery of bioactive agents like bixin.

Nanofibers composed of bixin-loaded polycaprolactone (Bix-PCL) have been developed and applied for the treatment of cutaneous wounds in diabetic mice. The formulation showed potential in promoting wound healing, highlighting the usefulness of bixin in skin regeneration applications.[65] Based on bixin content, two formulations were designed, Bix-PCL-1 (2.5% w/w bixin) and Bix-PCL-2 (12.5% w/w bixin) and subsequently evaluated via x-ray diffraction, thermal analysis, electrical conductivity, SEM and attenuated total reflectance infrared spectroscopy. In an in vitro drug release profile, initial burst release was observed during first 10 hours, 30% from Bix-PCL-1 and 40% from Bix-PCL-2 followed by the 100% slow and continuous release of bixin from nanofibers within 14 days. Results concluded that the Bix-PCL nanofibers are an auspicious technique for wound healing and also promote the bioaccessibility of bixin.[65]

A study was conducted to develop nanoencapsulated carotenoids, specifically lutein, using low molecular weight chitosan (LMWC) as the encapsulating agent. The nanoformulation aimed to enhance the stability, bioavailability, and controlled release of lutein for potential therapeutic applications.[66] LMWC served as a nanocarrier for lutein and aided in enhancing its bioavailability. The bioavailability of lutein-loaded LMWC nanoencapsules was prominently higher than the control. In another study,[67] lipid-based solid dispersions (LBSD) with incorporation of lycopene were prepared in order to enhance its oral bioavailability. Lycopene is a carotenoid with potent antioxidant and anti-cancerous activity. The designed formulation was compared with the commercially available product ie, Lycovit®. In conclusion, a clear difference between C_{max} and AUC existed. Moreover, oral bioavailability of lycopene was significantly improved in fasted pigs via the designed formulation (lycopene-loaded LBSD), in contrast to the commercial one.

Nano-Drug Delivery Strategies Promoting Bixin's Stability

In one of the study,[68] the stability of bixin was evaluated by incorporating it into the lipid-core nanocapsules. For this, a model system of water and ethanol (8:2) was employed and the stability of the designed formulation was tested during heating and photosensitization at (65–95°C) and (5–25°C), respectively. During photosensitization, activation energies were monitored for both free bixin and bixin-loaded lipid core nanocapsules with and without oxygen. Furthermore, activation energies for formulation and free bixin were calculated during heating as well. In conclusion, it was summarized that the stability of bixin in the designed formulation has been increased. Lobato et al performed a study,[69] to monitor the physical stability of bixin-loaded nanocapsules. The formulation was designed via the interfacial deposition technique by using poly-caprolactone (PCL). Five different formulations of bixin-loaded nanocapsules were prepared, attaining 11, 16, 37, 58 and 100 µg/mL concentrations of bixin. All formulations are kept in amber glasses under a temperature of 25 ± 1°C. After performing the characterization of the optimized formulation at predetermined time intervals for various parameters, it was evaluated that the bixin-loaded nanocapsules were physically stable for about 119 days at ambient temperature.

In a separate study,[70] bixin microparticles were designed via spray-drying technique, in which bixin was encapsulated in the galactomannan polymer. Various parameters, ie, physiochemical features, particle size, encapsulated efficiency, release profile and toxicological behavior were monitored. During examination, it was analyzed that the encapsulating efficiency of bixin is more than 90% with an average particle size of 5.55 ± 0.50 µm. After stability testing, it was confirmed that the microencapsulation contributes compelling stability for bixin.[70]

A study investigated the use of maltodextrin as an encapsulating agent for bixin, employing a freeze-drying technique to improve its stability and handling characteristics.[71] After that, for the sake of evaluating the influence of maltodextrin concentration and bixin-to-matrix ratio on the solubility and encapsulation efficiency of bixin, a preformed experimental design was employed. A 40% w/v concentration of maltodextrin and a 1:20 bixin-to-matrix ratio delivered the highest encapsulation efficiency. Meanwhile, the highest solubility was monitored at a 20% w/v concentration of maltodextrin and a 1:20 bixin-to-matrix ratio. As a result, the solubility and stability of the given compound improved after encapsulation.[71]

In a related study, a combination of modified starch and gelatin was used for the microencapsulation of bixin through the spray-drying method, aiming to enhance its stability and protect it from environmental degradation.[72] Both commercial and solvent extracts of annatto seed ie, bixin were employed for microencapsulation. Furthermore, encapsulating materials were utilized in different ratios, starch-to-gelatin (100:0 and 60:40) to produce microcapsules of bixin. The ratio of 60:40 provided the highest encapsulation performance. For commercial extract microcapsules 86.37% and for solvent extract microcapsules 86.18% encapsulation efficiency was recorded. In an in vitro release study, initial burst release followed by the controlled release of extracted substances was analyzed. Moreover, the encapsulated product is more stable against light, heat and oxygen in contrast to non-encapsulated material. Bixin has also been shown to function as a nano-dispersion former with notable antimicrobial activity against both gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Additionally, bixin-based nano-dispersions have been successfully loaded with amphotericin B, demonstrating their potential as drug delivery carriers.[73]

Design–Activity Relationship in Bixin Nanodelivery Systems

Different design parameters, ie, particle size, morphology, and type of carrier, strongly influenced the therapeutic performance of bixin. The amphiphilic nature of bixin encourages its integration into diverse nanodelivery platforms, ie, polymeric nanoparticles, lipid-based matrices and biopolymeric carriers. These design parameters directly influence solubility, stability, cellular uptake, and drug release behavior, which together influence the overall pharmacological activity of the formulation.[20]

Nanocarriers efficiently encapsulated the drug particles and small size of these nanocarriers facilitated the improved cellular uptake and bioavailability of the model drug. Improved therapeutic activity of bixin-loaded polymeric nanoparticles was observed in cigarette smoke-induced lung inflammation and oxidative stress in mice, in comparison with free bixin, emphasizing the importance of particle size.[16] Furthermore, lipid-based drug delivery platforms such as SLNs and NLCs not only support the enhanced cellular uptake and bioavailability but also deliver controlled drug release, improved stability and protection of bixin from photodegradation. Their lipophilic core promotes drug loading, while the amphiphilic surface facilitates interaction with biological membranes.[52]

Encapsulation techniques also critically influence the therapeutic activity of bixin, as they encapsulate the drug and prevent it from oxidative degradation, controlled release kinetics, and enhance pharmacological responses as compared to unencapsulated forms.[72]

Collectively, these observations highlight a clear design–activity relationship in bixin-based nanosystems. The pharmacokinetic behavior, drug release kinetics and biological performance are directly governed by the compositional optimization of nanoparticles. Therefore, maximizing the therapeutic potential of bixin is essential for future translational applications.

Leveraging Carotenoid-Based Nanocarriers to Enhance Bixin's Pharmacokinetic Properties

A comparative summary of nanodrug delivery systems applied to structurally related carotenoids such as β-carotene, lycopene, lutein, and astaxanthin have been provided to highlight formulation trends relevant to bixin (Table 1). These carotenoids share similar physicochemical limitations, including poor aqueous solubility, chemical instability, and low oral bioavailability. The table presents a range of delivery platforms, including oil-in-water nanoemulsions, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), liposomes, polymeric nanoparticles, and niosomes, along with their associated outcomes in terms of drug loading efficiency, antioxidant activity, cellular uptake, stability under physiological conditions, and enhanced bioavailability parameters such as increased area under the curve (AUC) and systemic absorption. This detailed comparison offers insight into the effectiveness of various nanocarriers in overcoming the inherent limitations of lipophilic compounds. Observations such as improved therapeutic response, sustained release

Table 1 Summary of Nanodrug Delivery Systems for Carotenoids Structurally Related to Bixin and Their Impact on Stability and Bioavailability

Incorporated Carotenoids	Delivery Systems	Research Outcomes	References
β-carotene	O/W Nanoemulsion	Increased bioactive agent stability. Improved bioaccessibility/bioavailability.	[74]
	Nano-liposomes	Increased drug loading and bioaccessibility.	[75]
	SLNs	1.92-fold increased AUC, improved bioavailability. Increased anti-cancerous activity.	[76]
		Better compound stability and cellular uptake.	[77]
	Polymeric/ Bio-polymeric NPs	Better drug release profile. Improved antioxidant activity.	[78]
	Niosomes	Better stability against light and temperature.	[79]
	NLCs	Effective encapsulation and enhanced stability.	[80]
		Increased antibacterial activity. Significantly enhanced antioxidant feature.	[81]

(Continued)

Table 1 (Continued).

Incorporated Carotenoids	Delivery Systems	Research Outcomes	References
Lycopene	Nano-liposomes	Improved antioxidant activity. Better bioavailability.	[82]
	SLNs	Highly stable after loading into nanocarriers.	[83]
	NLCs	Burst release followed by sustained drug release. Increased permeation and bioavailability.	[84]
		Improved stability. Prolonged drug release profile.	[85]
	Niosomes	Upgraded drug anti-cancerous activity. Elevated bioavailability.	[86]
	Bio-polymers NPs (dextran, chitosan and WPC)	Better encapsulation via WPC. Reduce degradation against heat and moisture.	[87]
	O/W Nanoemulsion	Formulation designed via sesame oil have improved stability and decreased degradation along with high degree of bioaccessibility.	[88]
Lutein	Polymeric NPs	High entrapment efficiency. Better stability and increased cellular uptake.	[89]
		Lutein-loaded ZNPs demonstrated good stability to be improved by 58% and encapsulation behavior.	[90]
		Lutein-loaded LMWC nanoencapsules presented 27.7% improved drug's bioavailability. Postprandial level of lutein increased in plasma, liver and eyes is 54.5%, 53.9% and 62.8%, respectively as compared to control.	[66]
	Nano-liposomes	PLL-modified lutein nanoliposomes shown improved entrapment, reduced degradation and enhanced bioavailability.	[91]
	Nanoemulsion	WPI-based formulation gave better physiochemical features along with efficient drug entrapment.	[92]
	Liposomes	Physical stability of lutein was monitored in stimulated GIT fluid. Fast drug release monitored for lutein in GIT fluid. Bioaccessibility of drug improved after loading into the nanocarrier.	[75]
Astaxanthin	O/W Nanoemulsion	Three oils (olive, flaxseed and corn oil) employed to develop nanoemulsion and all three of them exhibited enhanced bioaccessibility and accordingly, promoted the bioavailability.	[93]
	Polymeric nanospheres	Three different polymers (PCPLC, PB4, EC) were employed to encapsulate the astaxanthin. PCPLC gave better encapsulation of about 98% with loading of 40% (w/w). Moreover, minimal heat degradation and good dispersibility achieved via PCPLC derived nanospheres.	[94]
	Nanoliposomes	Reduced particle size with higher encapsulation entrapment. TGA proved the thermal stability of nanoliposomes, increased aqueous dispersibility of astaxanthin.	[95]
	Emulsions	AST-loaded emulsion promoted the physical and chemical stability but elevated temperature boosted the chemical degradation of incorporated drug.	[96]
	SLNs	Radiolabeled drug nanocarriers demonstrated the increased drug concentration in brain via intranasal route. It was observed that radiolabeled particles were 96–98% stable	[97]
	NLCs	Physical stability and organoleptic properties were improved.	[98]

Abbreviations: O/W, Oil-in-water; SLNs, Solid lipid nanoparticles; AUC, Area under the curve; NPs, Nanoparticles; NLCs, Nanostructured lipid carriers; WPC, whey protein concentrate; PLL, Poly-L-lysine; WPI, whey protein isolate; GIT, gastro-intestinal tract; PCPLC, poly (ethylene oxide)-4-methoxycinnamoylphthaloylchitosan; PB4, poly(vinylalcohol-co-vinyl-4-methoxycinnamate); EC, ethyl-cellulose; TGA, thermal gravimetric analysis; AST, Astaxanthin.

behavior, and enhanced biological performance support the relevance of these systems for bixin delivery. Additionally, the table illustrates how different polymers, surfactants, and encapsulation techniques contribute to the physicochemical enhancement of carotenoids, thereby providing a practical reference framework for designing advanced bixin-based formulations with improved therapeutic potential.

Amphiphilic Nature and Self-Assembly Behavior of Bixin

Recently in 2024, a study was conducted by Patra et al, which highlights the drug delivery potential of bixin.[20] The prime objective of the study is to investigate and report the first self-assembly of the bixin in aqueous liquids, thereby establishing its potential as a novel drug delivery system. Bixin is an apocarotenoid containing a long, 24-carbon polyene chain with nine conjugated double bonds. The molecule is characterized as a single-chain amphiphile because it possesses both a lipophilic (hydrophobic) long polyene chain and a polar (−COOH) head-group at one terminal, alongside a methyl ester group (-COOCH$_3$) at the other terminal. So, this molecule renders the self-assembly mechanism because the polar -COOH head-group is driven outward towards the aqueous environment, while the hydrophobic polyene chains pack tightly inwards, leading to the formation of a bilayer membrane, as verified via High Resolution Transmission Electron Microscopy (HRTEM) that confirmed a bilayer membrane morphology of vesicles, supporting its suitability for forming drug carriers. In conclusion, it was claimed that the spontaneous self-assembly of bixin in aqueous liquids provides significant benefits primarily related to drug delivery and enhanced therapeutic action. The self-assemblies of bixin facilitate the efficient drug entrapment and ensure controlled drug release; moreover, alone and in combination with curcumin, bixin's self-assemblies demonstrated remarkable antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*.

Conclusion

Bixin, a prominent apocarotenoid pigment derived from *B. orellana* seeds, has emerged as a multifaceted bioactive compound with significant therapeutic and industrial potential. This review summarizes the broad pharmacological spectrum of bixin, including its potent antioxidant, anti-inflammatory, anticancer, antimicrobial, neuroprotective, hepato-protective, nephroprotective, and photoprotective activities. Mechanistically, many of these effects are mediated through the NRF2 signaling pathway, STAT6 suppression, and ROS scavenging pathways central to oxidative stress mitigation and inflammation control. Preclinical studies have demonstrated bixin's potential in disease models such as multiple sclerosis, Alzheimer's disease, colorectal cancer, melanoma, ventilator-induced lung injury, cigarette smoke-induced inflammation, and age-related macular degeneration. Structurally, bixin possesses an amphiphilic nature due to its polar carboxylic and nonpolar methyl ester termini, enabling interactions with both aqueous and lipid environments. This amphiphilic property contributes to its biological versatility and supports its applicability in modern drug delivery systems. In addition, emerging data suggest its compatibility with modern drug delivery technologies. Despite its promising bioactivity, bixin faces certain limitations in terms of bioavailability, stability, and solubility, which restrict its clinical application. Recent advances in nanoparticle formulations, sustained-release systems, and polymer-based encapsulation strategies have shown promise in overcoming these challenges, thereby enhancing the compound's therapeutic index.

Importantly, this review presents a unique perspective by consolidating both the pharmacological potential and the drug delivery challenges of bixin, which are often underrepresented in the current literature. The review also integrates mechanistic insights and cross-disciplinary applications ranging from functional foods and cosmeceuticals to nanomedicines and photoprotection to provide a holistic understanding of bixin's potential. Future research should focus on detailed pharmacokinetics, toxicological profiling, structure-activity relationships (SAR), and clinical trials, which are critical to transitioning bixin from a phytochemical of interest to a clinically viable therapeutic agent. Furthermore, its possible role in combination therapies for inflammatory and oxidative stress–related disorders represents an area of emerging interest that warrants systematic exploration. With continued interdisciplinary research, bixin may eventually evolve from a traditional dye to a promising natural therapeutic candidate.

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Disclosure

The authors report no conflict of interest in this work.

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