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A REVIEW OF BIXIN BIOSYNTHESIS IN BIXA ORELLANA ROLE AS ANTIOXIDANT, ANTI-INFLAMMATORY, ANTICANCER, AND SKIN PROTECTING NATURAL PRODUCT EXTRACTED FROM BIXA ORELLANA L.

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ABSTRACT

Since long, medicinal plants or herbs are being used in different traditional treatment systems as therapeutic agents to treat a variety of illnesses. Bixa orellana L., an medicinal plant (Family: Bixaceae), is an Ayurvedic herb used to treat dyslipidemia, diarrhoea, and hepatitis since ancient times. *B. orellana* L., seeds contain an orange-red coloured component known as bixin *($C_{25}H_{30}O_4$)*, which constitutes 80% of the extract. Chemically, bixin is a natural apocarotenoid, biosynthesized through the oxidative degradation of C40 carotenoids. Bixin helps to regulate the Nrf2/MyD88/TLR4 and TGF-1/PPAR-/Smad3 pathways, which further give it antifibrosis, antioxidant, and anti-inflammatory properties. This current review article presents a comprehensive review of bixin as an anti-inflammatory, antioxidant, anticancer, and skin protecting natural product. In addition, the biosynthesis and molecular target of bixin, along with bixin extraction techniques, are also presented.

Bixa orellana (Family Bixaceae) is a neotropical fast growing perennial tree of great agro-industrial value because its seeds have a high carotenoid content, mainly bixin. It has been used since pre-colonial times as a culinary colorant and spice, and for healing purposes. It is currently used as a natural pigment in the food, in pharmaceutical, and cosmetic industries,

and it is commercially known as annatto. Recently, several studies have addressed the biological and medical properties of this natural pigment, both as potential source of new drugs or because its ingestion as a condiment or diet supplement may protect against several diseases. The most documented properties are anti-oxidative; but its anti-cancer, hypoglycemic, anti-biotic and anti-inflammatory properties are also being studied. Bixin's pathway elucidation and its regulation mechanisms are critical to improve the produce of this important carotenoid. Even though the bixin pathway has been established, the regulation of the genes involved in bixin production remains largely unknown. Our laboratory recently published B. orellana's transcriptome and we have identified most of its MEP (methyl-D-erythritol 4-phosphate) and carotenoid pathway genes. Annatto is a potential source of new drugs and can be a valuable nutraceutical supplement. However, its nutritional and healing properties require further study.

KEYWORDS: Bixin, Bixa Orellana, anti-inflammatory, anticancer, skin protecting, apocarotenoids, bixin biosynthesis, anti-cancer, antigenotoxic, antioxidant, hypoglycemic.

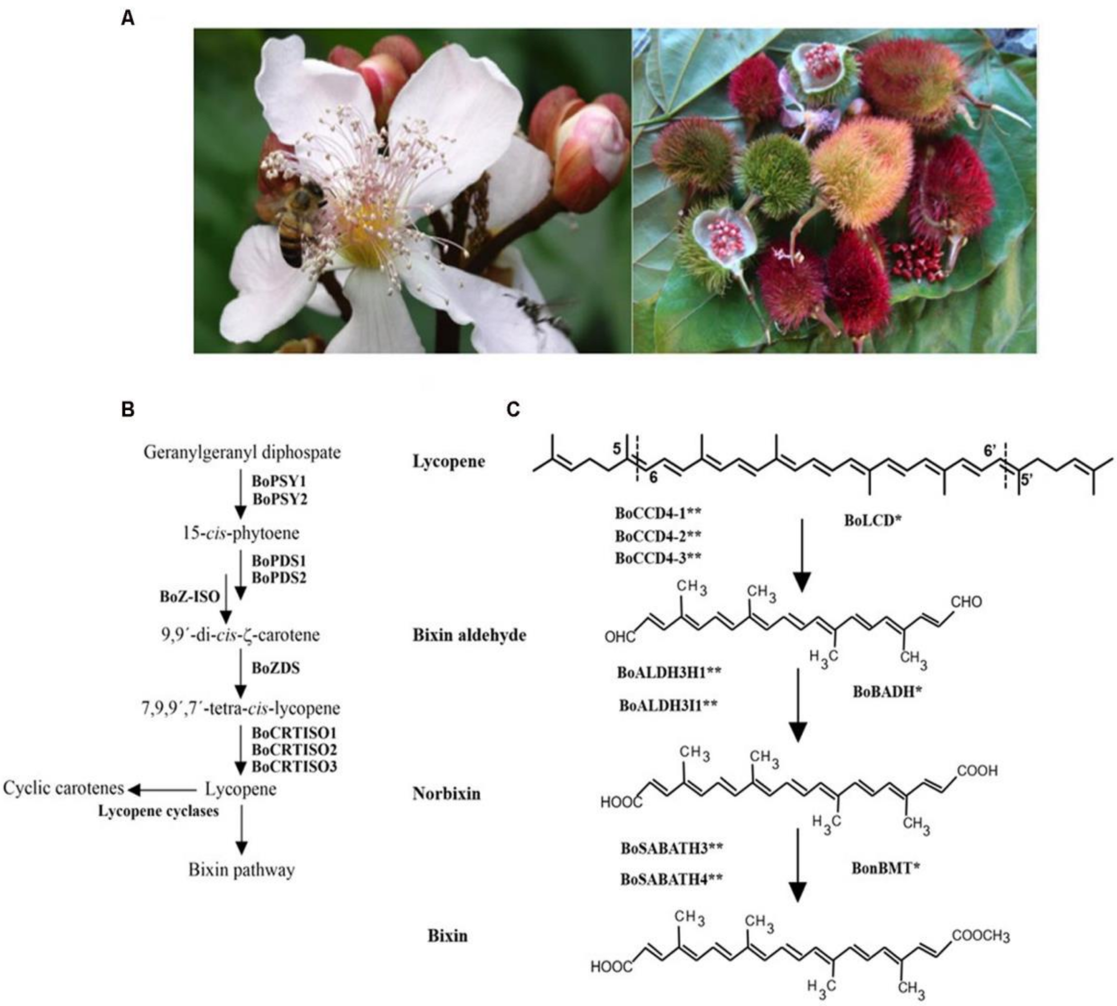
Graphical abstract

INTRODUCTION

A Brazilian medicinal native plant, *Bixa orellana* L., is also well-known as "achiote" in Mexico, "colorau" and "urucum" in Brazil, and in the United States, it is named "annatto".[1] Indigenous people use the pulp from the seeds of the plant, *B. orellana*, sometimes known as the "lipstick tree," therapeutically to improve the appearance of lips.[2] *B. orellana* L., seeds are generally used in traditional medicine to treat dyslipidemia, diarrhoea, and hepatitis. Among local communities, *B. orellana* L., seeds are popular for their use as a laxative as well as a condiment, hypotensive, cardiotonic, expectorant, and antibiotic.[3],[4],[5] In addition, it has an anti-inflammatory activity for wounds and bruises, and it has been used for the treatment of bronchitis and febrifuge, and soft drinks are prepared from the pulp of the seeds.[3,6] Phytochemical analysis conducted in the past revealed that *B. orellana* L. seeds have insect repellent, antileishmanial, antioxidant, antihyperlipidemia, antimalarial, cytotoxic, hypoglycemic, and allergenic activity.[6] Apart from these, a number of other activities of *B. orellana* L. seeds have been tested in different test models, but the results were not satisfactory. Pharmacological potential of *B. orellana* L. seeds is presented in Table 1. While the leaf infusion has provided promising results for its effective use in the treatment of eye inflammation, sore throat, and bronchitis.[15]

Aside from ethnomedicinal applications, *B. orellana* is economically significant because of a red dye extracted from its seeds.[16] This dye is broadly used in the pharmaceutical, food, ornamental,and textile industries, accounting for approximately 70% of all natural colouring agents consumed globally.[15,17] This red color is proportional to the percentage of soluble annatto extract (E160b), and the main orange-red coloured component is called bixin *($C_{25}H_{30}O_4$)*, which accounts for 80% of the extract. This natural colourant was first isolated in 1875 from the seeds of *B. orellana* L., and its complete chemical structure was first discovered in 1961 (Fig. 1). The carotenoid content of this plant, especially bixin, has played a major role in developing medicinal uses and industrial uses for annatto seeds, while chemically, bixin is a type of apocarotenoid.[18] The oxidative degradation of C40 carotenoids is involved in the biosysnthesis of bixin (an apocarotenoid). The molecular weight of bixin is 394.51 g/mol, and it has a 25-carbon open chain structure with a methyl ester group and carboxylic acid present at the end. Bixin occurs naturally as the 16-(cis) isomer, but it isomerizes during the extraction process into the 16-(trans) form, called isobixin.[19] Bixin is biosynthesized through the oxidative degradation of C40 carotenoids. The Food and Drug Administration (FDA) has given bixin its approval as an edible colourant to be used in processed bakery products, ice cream, butter, cheeses, cereals, margarine, meats, and also in cosmetics.[18,20] Long before FDA approval, it was considered as a dye in foods, such as milk products and beverages, food supplements, bakery products, and vegetable oils. The most common ethnomedicinal application of bixin among traditional medicine practitioners has been to treat pain problems and diabetes.[15,21] Researchers have also worked with bixin (the major compound of *B. orellana* L., seeds), which was found to exhibit anti-inflammatory, antioxidant, lung protective, and hepatoprotective properties. Furthermore, inflammatory responses and oxidative stress produced due to high-calorie meals can be reduced by taking bixin supplementation.[17]

We searched the available data resources, such as PubMed, Scopus, Google Scholar, and Web of Science, for this review to accumulate all the information related to the anticancer, antioxidant, anti-inflammatory, and skin-protecting potential of bixin. This current review article presents a comprehensive review of bixin as an anti-inflammatory, antioxidant, anticancer, and skin protecting natural products. In addition, the biosynthetic pathway of Bixin in *B. orellana* L., and molecular target of bixin, along with the efficient way to extract bixin from *B. orellana* L., seeds, are also presented.



Bixin Biosynthesis in Bixa Orellana

Bixin, extracted from the seeds of Bixa orellana L., is a natural compound known for its diverse health benefits, including antioxidant, anti-inflammatory, anticancer, and skin-protecting properties. Below, I provide a detailed overview of these roles and the biosynthesis of bixin.

Antioxidant properties

Bixin is a potent antioxidant due to its ability to neutralize reactive oxygen species (ROS). It effectively scavenges free radicals, thereby protecting cells from oxidative damage. This antioxidative action is beneficial in preventing lipid peroxidation and maintaining cellular integrity. Studies have demonstrated bixin’s ability to reduce oxidative stress in various models, including protection against cigarette smoke-induced lung inflammation in mice.

Anti-inflammatory effects

Bixin exhibits significant anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and modulating inflammatory pathways. This makes it valuable in treating chronic inflammatory conditions. For instance, bixin-loaded nanoparticles have been shown to reduce acute lung inflammation and oxidative stress, highlighting its potential in managing inflammatory diseases.

Anticancer activity

Bixin has demonstrated promising anticancer properties. It can induce apoptosis (Programmed cell death) in cancer cells and inhibit their proliferation. Research on hepatocellular carcinoma and other cancer cell lines has shown that bixin can trigger apoptosis and interfere with cell cycle progression, suggesting its potential as a therapeutic agent in cancer treatment.

Skin protection

Bixin offers protection against skin damage, particularly from ultraviolet (UV) radiation. By activating the NRF2 pathway, bixin enhances the skin's defense mechanisms against oxidative stress and photodamage. It has been found to protect against UV-induced skin damage and reduce signs of photoaging, such as hair graying, in animal models. This photoprotective effect underscores bixin’s potential in skincare applications (MDPI) (Frontiers).

Steps in bixin biosynthesis

Lycopene cleavage

The process begins with the carotenoid lycopene, which is cleaved by carotenoid cleavage dioxygenases (CCDs). These enzymes specifically target the double bonds in lycopene, resulting in the formation of bixin aldehyde.

Enzymes involved: BoCCD4 (Carotenoid Cleavage Dioxygenase 4).

Conversion to norbixin

The bixin aldehyde is then oxidized by aldehyde dehydrogenases to form norbixin. This step is crucial as it prepares the molecule for the final methylation step.

Enzymes involved: BoALDH (Aldehyde Dehydrogenase).

Methylation to bixin

Norbixin undergoes methylation by specific methyltransferases to produce bixin. This methylation process adds a methyl group to the carboxyl group of norbixin, converting it into bixin.

Enzymes involved: BoBMT (Bixin Methyltransferase)

Plant carotenoids have a crucial role in photosynthesis helping to collect light and conferring protection against its excess. Carotenoids are also important precursors of bioactive compounds, such as apocarotenoids which are important in several physiological processes, such as retinol in humans and abscisic acid in plants. Most apocarotenoids are carotenoid degradation products bio-catalyzed by carotenoid cleavage oxygenase enzymes (CCDs). Similar to others apocarotenoids pathways, the biosynthesis pathway of bixin, elucidated in the early 2000 s, involves carotenoid cleavage by CCDs enzymes; the first step is lycopene cleavage in 5–6 and 5′–6′ double bonds (Figures 1B,C).

Based on expressed sequences tags (ESTs) library from immature seeds, the first bixin biosynthesis pathway was proposed by Jako et al. (2002). They found cluster of genes related to dioxygenase, aldehyde dehydrogenase and methyl transferase genes with high number of ESTs, suggesting that bixin pathway should be similar to abscisic acid pathway and that bixin’s precursor is a C40 carotenoid, probably lycopene, which is converted to bixin by dioxygenase, aldehyde dehydrogenase and methyl transferase genes (Jako et al., 2002). Additionally, they found cluster of genes expressed in immature seeds, where the main production of bixin takes place, related to 1-Deoxy-D-xylulose-5-phosphate synthase (DXS), 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), 4-Hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (HDS) and 4-Hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR) from methyl-D-erythritol 4-phosphate (MEP) pathway and Phytoene synthase (PSY), Phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS) from carotenoid pathway.

Simultaneously, Bouvier et al. (2003) proposed a similar bixin pathway; they hypothesized that bixin pathway should be like saffron pigment crocetin and that the reaction could implicate a dioxygenase, an aldehyde dehydrogenase, and a methyltransferase enzyme that converted lycopene to bixin in serial step reactions (Bouvier et al., 2003) (Figure 1C). They identified and isolated a family 4 dioxygenase (BoLCD), aldehyde dehydrogenase (BoBADH), and methyltransferase (BonBMT) genes. These genes were introduced into engineered Escherichi coli lycopene producer; transformed bacteria were able to convert lycopene to bixin (Bouvier et al., 2003).

Although bixin pathway has been established, the expression regulation of genes involved in bixin production is unknown, perhaps because the MEP and carotenoids pathways genes, as well as the transcription factor that regulate them remain unaddressed. Recently, during the *B. orellana* transcriptome analysis, the authors identified most of its MEP and carotenoid pathway genes for this plant (Cárdenas-Conejo et al., 2015). Interestingly, a quantitative real time PCR (qRT-PCR) showed that BoDXS2a, BoPDS1 and BoZDS genes were overexpressed in immature seeds, where most bixin is produced, as compared to leaves, whereas carotenoids pathway genes downstream of lycopene were not overexpressed (Cárdenas-Conejo et al., 2015).

Surprisingly, the three genes identified by Bouvier et al. (2003) were not present in *B. orellana* transcriptome, and may have been misplaced in the original study (Cárdenas-Conejo et al., 2015). Based on subcellular localization prediction, function of homologous proteins and qRT-PCR quantification, Cárdenas-Conejo et al. (2015) proposed a new set of genes involved in the conversion of lycopene into bixin (Figure 1C); enzymatic activities for this new set of genes need to be characterized.

The enzymes involved in bixin production are present in most plants. Since these enzymes play other important metabolic roles, finding other plants with the ability to produce bixin is not surprising. *Crocus sativus*, *Vitis vinifera*, and *Costus pictus* produce bixin in detectable levels (Siva et al., 2010; Annadurai et al., 2012). The high quantity of bixin produced in B. orellana immature seeds is likely due to the gene expression synchronization of the expression of the genes involved in bixin production, including MEP and carotenoid pathways genes.

Cárdenas-Conejo et al. (2015), proposed an hypothetical model for bixin production in B. orellana immature seeds involving the coordinated expression of MEP, carotenoid and bixin pathway genes: (1) MEP genes involved in generation of carotenoids precursors, such as BoDXS2a, BoDXR and BoHDR are induced to produce carotenoids in non-photosynthetic tissue. Enzymes from the DXS2 clade, but not from the DXS1 or DXS3 clades, are involved in carotenoid and apocarotenoid accumulation in non-photosynthetic tissues (Floss et al., 2008; Peng et al., 2013; Saladié et al., 2014). (2) Similar to the tomato ripping process, lycopene cyclase genes from B. orellana are turned off, thus blocking metabolic flow toward cyclic carotenoids down-stream of lycopene. The low concentrations of cyclic carotenoids induce the expression of BoPDS1 and BoZDS and promote lycopene production. (3) The bixin pathway genes are then turned on, leading to the conversion of lycopene into bixin.

Full elucidation of the molecular mechanisms that govern bixin production will help understand the mechanisms responsible for the variation of bixin accumulation in B. orellana varieties and identify the candidate genes for genetic improvement of this plant to enhance the bixin production.

Molecular targets of bixin

Bixin, a carotenoid found in the seeds of the annatto plant (Bixa orellana), has been studied for its various biological activities and potential health benefits. Research has identified several molecular targets through which bixin exerts its effects. Here are some key molecular targets and pathways influenced by bixin.

Antioxidant activity: Bixin is known for its strong antioxidant properties. It scavenges free radicals and reduces oxidative stress by interacting with reactive oxygen species (ROS). This antioxidant effect is crucial in protecting cells from oxidative damage, which is linked to various chronic diseases.

Anti-inflammatory effects: Bixin modulates inflammatory responses by targeting various signaling pathways:

NF-κB pathway: Bixin inhibits the activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), a key transcription factor involved in the inflammatory response. By preventing NF-κB activation, bixin reduces the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.

COX-2 and iNOS expression: Bixin downregulates the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), enzymes that play significant roles in inflammation and pain.

Anti-cancer activity: Bixin has been shown to have potential anti-cancer properties through various mechanisms.

Apoptosis induction: Bixin induces apoptosis (programmed cell death) in cancer cells by modulating the expression of pro-apoptotic and anti-apoptotic proteins such as Bax and Bcl-2, and activating caspase enzymes.

Cell Cycle arrest: It can cause cell cycle arrest in cancer cells, thereby inhibiting their proliferation. This is achieved by influencing cell cycle regulatory proteins like cyclins and cyclin-Dependent kinases (CDKs).

Lipid metabolism: Bixin affects lipid metabolism and can help in the prevention of atherosclerosis:

PPAR pathways: Bixin activates peroxisome proliferator-activated receptors (PPARs), particularly PPAR-α and PPAR-γ. These nuclear receptors play key roles in regulating lipid metabolism, glucose homeostasis, and anti-inflammatory responses.

Neuroprotective effects: Bixin has shown promise in neuroprotection through various mechanisms:

Mitigation of neuroinflammation: By inhibiting inflammatory pathways and reducing oxidative stress, bixin can protect neurons from damage.

Promotion of neurogenesis: Some studies suggest that bixin may promote the survival and differentiation of neuronal cells.

Modulation of enzyme activity: Bixin can inhibit or modulate the activity of certain enzymes involved in disease processes. For instance, its inhibition of matrix metalloproteinases (MMPs) is relevant in the context of cancer metastasis and tissue remodeling.

Bixin acts on diverse molecular adaptor molecules in the human body to exert its biological properties. Bixin shows anti-inflammatory, antoxidant and antifibrosis properties by the regulation of TLR4/Nrf2/MyD88 and TGF-β1/PPAR-γ/Smad3 pathways.[26] Phase II detoxification, inflammatory signalling, DNA repair, and the antioxidant response are only a few of the important cellular coping mechanisms regulated by means of the transcription factor Nrf2 (nuclear-factor erythroid 2-related factor 2).

Antioxidant activity

The extract of annatto seeds contains primarily bixin and several other polyphenolic compounds that are responsible for antioxidant activity. In a research study, Kurniawati et al., (2010) determined the antioxidant activity by means of the DPPH (1–1 diphynilpicrylhidrazil) method, *in vitro*.[40] They have found that the determined antioxidant activity of bixin exhibited an IC_{50} value of 548.5 ± 20.0 ppm. In a recent work, González-Araúz et al., (2022) determined and evaluated. Bixin exhibits strong antioxidant activity, which is primarily due to its ability to scavenge free radicals and reactive oxygen species (ROS). This antioxidant effect is crucial for protecting cells from oxidative stress, which can lead to various chronic diseases. Studies have shown that bixin can inhibit lipid peroxidation and enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase.

Anti-inflammatory activity

According to several studies, both *in vitro* and *in vivo*, bixin has potent anti-inflammatory activity. Bixin supplementation reduces inflammatory responses in the lungs, skin, and heart of animals by suppressing oxidative stress.[44] Bixin exhibits remarkable inhibitory activity against bradykinin-induced inflammation. Furthermore, the activation of the antioxidant transcription factor Nrf2 activity *via* a number of studies has demonstrated bixin's anti-inflammatory properties.[27]

Inflammation is a biological response to harmful stimuli, but chronic inflammation can lead to numerous health issues, including autoimmune diseases and cancer. Bixin has been found to possess significant anti-inflammatory properties. It can modulate the inflammatory response by inhibiting the production of pro-inflammatory cytokines and enzymes such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and cyclooxygenase-2 (COX-2). These effects make bixin a potential therapeutic agent for inflammatory diseases.

Anticancer activity

The most known type of hepatic cancer is hepatocellular carcinoma (HCC), and it is resistant to standard therapy treatment. Due to its potent anticancer capabilities, bixin has recently attracted the attention of scientists. The number of Hep3B cells is significantly reduced by bixin treatment, and while changes in nuclear and cellular morphology were observed during morphological analysis, these changes can activate different biological processes in apoptosis as determined *via* annexin.

The anticancer potential of bixin is attributed to its ability to induce apoptosis (programmed cell death) and inhibit cell proliferation in various cancer cell lines. Bixin has been shown to interfere with multiple signaling pathways involved in cancer progression, such as the NF-κB pathway, which is known to regulate the expression of genes involved in cell survival and proliferation. Additionally, bixin can arrest the cell cycle and reduce the invasiveness and metastasis of cancer cells, making it a promising compound in cancer therapy.

Skin protection

UV radiation from the sun can cause premature ageing, acute photodamage, and skin cancer; the main causes of all of these are neuroendocrine, oxidative, and inflammatory stress and UV-induced genotoxicity.[55] UVB-containing ultraviolet radiation is important in the pathogenesis of cutaneous cancer and the premature ageing of the skin. Excessive UVB exposure can result in DNA mutations in the skin. UVB is penetrated in the basal cells of the skin epidermis when skin cells are directly exposed. Bixin also demonstrates skin-protecting properties, which are particularly valuable in the context of photoprotection. It can absorb UV radiation, thereby preventing UV-induced damage such as erythema (skin redness) and photoaging. Furthermore, its antioxidant properties help neutralize ROS generated by UV exposure, protecting skin cells from oxidative damage. Bixin has been incorporated into various cosmetic formulations to enhance skin health and prevent premature aging.

Applications of bixa orellana in medicine

Bixa orellana has been extensively used since pre-hispanic times in America as a remedy for different illness. Now a days achiote trees are still used in many communities as a source of treatment for many diseases. During the XVII and XVIII Centuries, it spread widely to countries in Asia and Africa, where it also became part of the ethnobotanical cultural heritage. Although many of these properties have not been studied by modern science, the similarities of the uses given by different cultures could credit certain effectiveness, and shows the relevance of the studying its active principles. Ethnobotanical researchers have documented the use of different parts of the plant, especially leaves, seeds and roots (Kumar et al., 2009; Dike et al., 2012). In Southern Mexico it is used for smallpox and other rashes. It is also used in digestive illness, such as diarrhea, abdominal pain, indigestion and dysentery.

Other uses include headaches and sore throat, as abortive, to cure urinary illness and against gonorrhea, and as an abortive agent (Ini-Unam, 2009). In Nigeria it is used against malaria, as an antiseptic and anti-bacterial agent, and against rheumatism (Dike et al., 2012). Similar effects have been documented in Brazil, Peru, Colombia, and other countries in Central America (Vilar Dde et al., 2014) and India (Kumar et al., 2009). Modern science has studied just a few of the healing properties attributed to this species, generally using a mixture of compounds extracted from different parts of the plants.

The most documented effect of bixin in medicine is its antioxidant activity. In vitro experiments have shown that seed extracts have a high capacity to scavenge reactive oxygen species (ROS), which correlate to bixin concentration in the extracts (Campos et al., 2011). The possible mechanism for this effect is the electron transfer allowed by the double bonds found in this apocarotenoid or the hydrogen abstraction from carotenoid molecule functioning this way as a chain breaking antioxidant (Junior et al., 2005; dos Santos et al., 2012). The authors concluded that this great scavenging capacity could have clinical applications because bixin is capable of acting as an antioxidant by intercepting free radicals generated by commonly used chemotherapeutic drugs. The protective effect of seed extracts administered to cells and animals treated with cisplatin (cis-diamminedichloroplatinum II), a potent antitumoral agent with important side effects, has been documented (Silva et al., 2001; Rios et al., 2009). Important antigenotoxic effects were observed as a reduction of chromosomal aberration, ROS generation, lipid peroxidation, and inhibition of renal glutathione depletion. The effect is observed under a non-toxic dose and is a bixin concentration-dependent manner. Junior et al. (2005) reported not only a protective effect against chemotherapy, but also an anti-mutagenic effect on cells subjected to ultraviolet light. Other research papers also document anti-cancer effects of achiote seeds attributed to compounds such as geranylgeraniol, squalene and beta-sistosterol (Kumar et al., 2009). Tibodeau and Isham (2010) suggest that the anticancer effect could be possible because cis-bixin exerts its cytotoxic effects via imposition of cellular ROS mediated, at least in part, by inhibition of the thioredoxin = thioredoxin reductase redox pathway. Another possible use of achiote is in the prevention of diabetic complications due to oxidative stress. Rossoni-Júnior et al. (2012) showed that that supplementation with beta carotene and annatto is able to modulate the production of reactive species in diabetic animals.

Other medical uses of Bixa orellana might be re associated to other bioactive metabolites present both in leaves and seeds. A recent study validated the anti-inflammatory effect of this plant. Acute inflammation by injection of histamine in rats was inhibited by oral administration of leaf extracts, comparable to loratadine's effect. A reduction on vascular permeability was observed as a result of reduced expression of biochemical mediators such as nitrogen oxide and VEGF (vascular endothelium growth factor) (Yong et al., 2013).

In an experiment using tocotrienol extract from achiote to prevent osteoporosis, rats with testosterone deficiencies were treated with achiote seed extract. These rats showed less bone damage as compared to not-treated controls, and an increased expression of bone formation genes (Chin and Ima-Nirwana, 2014).

The antimicrobial activity of ethanol extracts of Bixa orellana leaves and seeds was tested in vitro on seven common microorganisms: Staphylococcus aureus, Staphylococcus pyogenes, Salmonella typhi, Escherichi coli, Candida albicans, Bacillus subtilis and Pseudomonas aeruginosa. Annatto extract inhibited growth of both fungi and bacteria. The effects were slightly lower compared to gentamicin for bacteria and nystatin for fungi (Fleischer et al., 2003). The effect of leaves and root alcoholic extracts on a resistant strain of N. gonorrhoeae was also tested in vitro showing an important growth inhibition, which was greater with leaf extracts (Cáceres et al., 1995).

Giorgi et al. (2013) documented the repellent efficiency of seed extracts using hexane, ethanol, and ethanol/water as solvents. They found repellence activity against Aedes aegiptii mosquito ranging from 22 to 90%, being the hexane extract at high concentrations (113.8 mg/ml) the most effective treatment.

Bixa orellana is a potential source of new drugs for a variety of conditions, due to the high concentration of carotenoid derived compounds, and probably because of the presence of other metabolites and peptides, some of them still uncharacterized. More pharmacological studies of this promising plant are needed before it can be used in modern medicine.

Dietary contribution

Achiote was used as a coloring agent in pre-hisipanc Mayan religious ceremonies and has been used since to color and flavor certain traditional dishes. In Yucatán, Mexico, the pigment is widely used in its internationally recognized local gastronomy. Achiote seeds contribute to human diet in Mexico and other American countries, and achiote pigments are distributed worldwide. Little is known about its protein and peptidic content, and still less about the biological functions of these molecules which could make the consumption of this natural product even more attractive (Coronado-Cáceres et al., 2014). A potential value of this product is the antioxidative function that could reduce the damage caused by free radicals, and be useful in cancer prevention (Reddy et al., 2005). Preliminary studies in our laboratory have given us clues about certain achiote peptides that could be established them as new nutraceutical cancer preventives against cancer (Coronado-Cáceres et al., 2014).

Carotenoids that have antioxidative effects have been identified in achiote; it has also been reported that the ingestion of this condiment reduces triglycerides in plasma (Kiokias and Gordon, 2003). It is well known that a key element in the development of diabetic complications is oxidative stress (Rossoni-Júnior et al., 2012). Levy et al. (1997) found in a study with a group of volunteers that after ingesting a single dose of 1 ml of a commercial annatto food colorant, bixin levels reached high concentrations in human plasma and were completely cleared in 8 h. Thus, the bixin present in processed foods may be an important nutritional factor that can promote human health. A study using carotenoid mixture found that they have antioxidative and anticarcinogenic effects (Reddy et al., 2005). However, more accurate studies need to be developed to demonstrate the specific effect of achiote pigments.

Interestingly, these pigments were found to have hypoglycemic effect using dogs, rats and human volunteers as experimental models (Fernandes et al., 2002; Junior et al., 2005; Russell et al., 2005). Thus annatto extract may have therapeutic potential for diabetes.

Mechanisms of action

Antioxidant mechanism: Bixin donates electrons to neutralize free radicals, thus preventing oxidative damage to lipids, proteins, and DNA.

Anti-inflammatory mechanism: It inhibits key inflammatory mediators and enzymes, reducing the overall inflammatory response.

Anticancer mechanism: Bixin induces apoptosis and cell cycle arrest in cancer cells, and modulates signaling pathways crucial for cancer cell survival and proliferation.

Skin protection mechanism: It absorbs UV radiation and neutralizes ROS, protecting skin cells from UV-induced damage.

CONCLUSION

The seeds of *Bixa orellana* L., sometimes known as annatto, contain the carotenoid bixin, which has substantial anticancer properties. What chemical mechanisms underlie bixin-induced apoptosis is not yet known, though. Bixin is a colourant broadly used in the foods, cosmetics, pharmaceuticals, and textile industries for the production of processed bakery products; butter, cheese, cereals, ice cream, margarine, meat products, dietary supplements, vegetable oils, and beverages. Bixin from Bixa orellana L. is a versatile natural compound with significant antioxidant, anti-inflammatory, anticancer, and skin-protecting properties. Its biosynthesis involves a series of enzymatic reactions transforming lycopene into bixin, highlighting the intricate biochemical pathways in plant secondary metabolism. Further research into bixin's mechanisms and applications could enhance its therapeutic potential in various fields of health and medicine.

Antioxidant effects of bixin and other achiote compounds have been demonstrated, so its consumption, either as a pigment or spice may provide health benefits. Other properties such as hypoglucemic and anticancer activities are being studied. Preliminary investigations in our laboratory with active peptides from seeds also suggest an effect in cancerous tumors (Coronado-Cáceres et al., 2014). It is important to promote the intake of achiote seeds and pigment in the diet for its medical, nutraceutical and nutritional potential values, as well as to promote its cultivation and production. Elucidation of bixin synthesis in achiote, and metabolite profiles are important research topics contributing to increase produce and use to promote human health. Further studies are still needed before bixin and other achiote compounds can be used extensively by modern medicine.

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