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REVIEW ARTICLE

Unveiling the multifaceted attributes of *Bixa orellana*: An insight into its chemical composition, industrial application and cultural significance

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Abstract

Bixa orellana L., commonly known as annatto or the lipstick tree, is an evergreen shrub from the Bixaceae family. Native to tropical regions of Central and South America, it is now cultivated globally. Renowned for its striking red flowers and spiny fruits, *B. orellana* seeds produce annatto, a natural dye rich in bixin and norbixin pigments. These water- and oil-soluble carotenoids are widely used as natural food colorants, especially with the rising demand for synthetic dye alternatives. Beyond its industrial applications in food, cosmetics, leather and solar cells, *B. orellana* has traditional medicinal uses for treating conditions like gonorrhea, asthma and sore throats. Its bioactive components, including carotenoids and essential oils, offer antioxidant and hypoglycemic properties. This review highlights the pharmacological activities, phytochemical composition and industrial relevance of *B. orellana*, showcasing its diverse economic, botanical and cultural significance.

Keywords

annatto; *Bixa orellana* L.; bixin; natural dye; norbixin

Introduction

Bixa orellana is a member of the Bixaceae family, which is made up of just one genus, Bixa. There are only five known species in this genus. The most notable of these is *B. orellana*, an evergreen shrub that is prized for its economic significance as well as its vivid red flowers and ornamental spiky fruits (1). Although it is native to tropical regions of Central and South America, *B. orellana* is an evergreen shrub that is grown around the world, including India (2).

Its outer seed extract is utilized as a natural food colouring agent due to the pigments bixin and norbixin and yield a dye named annatto and hence the plant is commonly called as "Annatto" and “Achiote” within the food sector. Bixin dissolves in fats, while norbixin dissolves in water. Depending on the extraction method, solvent and temperature, achiote can yield both water-soluble and oil-soluble colorants. This versatility makes achiote a highly valuable plant source for natural colorants (3). Moreover, they possess medicinal attributes, traditionally employed to address ailments such as

gonorrhoea and asthma and historically used as a gargle to alleviate sore throats (4). Bixin is a dicarboxylic monomethyl ester apocarotenoid pigment that confers orange-red color in the *B. orellana* seed. Plant secondary metabolites have enormous potential for use in nutrition and medicine. Limited studies and genetic improvement have been done on many plant species used to produce secondary metabolites that are essential parts of human food, animal feed, pharmaceuticals, biopesticides and bioherbicides. Due to its high bixin content, this tropical perennial and ligneous plant is very interesting to the agroindustry sector. South America is the origin of the *B. orellana,* also known as annatto. In pre-Colombian times, *B. orellana* was used as a cosmetic and traditional food ingredient.

Annatto dye is obtained from its seeds and its use has been stimulated by the prohibition of synthetic dyes in cosmetics and food, since it is one of the few accepted by the World Health Organization (WHO) for be non-toxic and do not appear to change the value of the food. It is estimated that 70 % of all natural dyes consumed in the world are derived from *B. orellana* dye. The ethnomedicinal use of the species is also found in communities in various countries, such as antidiarrheal, antipyretic, antidiabetic, hypotensive, expectorant and aphrodisiac a tivity, among others (5). In Trinidad and Tobago, popular veterinary use in dogs is also registered (6). Different parts of the plant can be used for this purpose, which includes the leaves and it is common for the filters to be used to treat health problems, more specifically for skin problems, antipyretic activity, diuretic action, as well as it can also be used to treat pain, gastrointestinal, respiratory and liver disorders, sexually transmitted diseases and snake bites (5). In addition, other preparations such as infusions and decoctions can also be used medicinally, such as for ocular inflammation and antiemetic therapy during pregnancy.

B. orellana is named in honour of Francisco Orellana, the first European to navigate the Amazon (7). It holds significant commercial value as it is cultivated for its natural dye, annatto, derived from the arils of its seeds. The pericarp of the seeds, the layer surrounding them, contains colors with broad industrial applications and garners commercial interest (8). The annatto seeds comprise an inner seed consisting of a shelled kernel containing oils, waxy substances, mineral ash and alkaloid compounds, along with a peel composed of cellulose and tannins and an outer covering containing pigments, moisture and a small quantity of oils (9 - 11).

Ethnobotanical use

Traditional medicine uses *B. orellana* extensively for the prevention and treatment of a wide range of illnesses, including jaundice, gonorrhoea, blood problems, fever, epilepsy and dysentery. The leaves in Ngaoundere, Cameroon, *B. orellana* are abundantly accessible and have long been utilized by this community to relieve joint pain, jaundice, fever and gastrointestinal pain. They are also used for the treatment of asthma and traditionally used as a gargle for sore throats. The bark and root are used for the fever. The leaves of *B. orellana* are used to cure snakebites, jaundice, diabetes and hypertension. The leaves of *B. orellana* possess anti-microbial, antifungal, anti-leishmanial, anti-inflammatory, analgesic and anti-convulsion activity (13) (Fig. 1).

Fig. 1. a) Plants of Annatto, b) Fruits, c) Capsule and seeds, d) Seeds.

Traditional knowledge of Bixa orellana

According to traditional knowledge, *B. orellana* has been used by various native peoples of the Americas for body painting and material purposes, as well as food coloring, or for use in religious and spiritual ceremonies and medicinal purposes (14). The diverse array of medicinal benefits of *B. orellana*, as summarized in Table 1 (15-29). Many aboriginal people use *B. orellana* for dyeing, in which the dye is obtained naturally as a mixture and is used to color pottery and other household vessels. Additionally, in addition to all the previously mentioned, many people also use *B. orellana* to protect themselves from mosquitoes present in the forests and ultraviolet radiation, while the phloem provides fibers for rough twine. The pulp, including the seed, is used as febrifuge and for soft drinks. In addition, it can generate valuable dyeing substances such as yellow (orellin) and red (bixin), which in turn constitutes a crystallized active product. In the food industry, *B. orellana* is used to color sauces, bakery products, cheese, macaroni, as well as juices, ice creams, soups, margarine, butter, mayonnaise, mustard and sausage, where it is commonly called “do reino” (from the kingdom), from the Netherlands (30). The medicinal use includes the treatment of various disorders of the human and veterinary organism, so that different parts of the plant can be used. The seeds have been used as a condiment, as well as a hypotensive, laxative, expectorant, antibiotic and cardiotonic. Furthermore, it possesses anti-inflammatory activity for wounds and bruises, besides to be used for the treatment of wound healings and bronchitis (30). The seeds are also used as an antidote for poisoning by *Manihot esculenta* (yucca, yuca brava, bitter yucca, sour yucca) and to prevent scars left by smallpox on the epidermis (31, 13). In Brazil, the seeds can be used to treat anemia, bronchitis and control cholesterol levels (32). Traditionally, the pulp of the fruits is applied to burns to prevent the formation of blisters and sores, while the powder resulting from crushing the seeds has been used as an aphrodisiac. Other properties related to the seeds are the following: antipyretic, laxative, antimalarial, antidiabetic, antidiarrheal, analgesic and treatment of respiratory problems. For the roots, antidiarrheal, antigonorrheal, anthelmintic activities and for the treatment of hepatic and respiratory disorders were reported. The fruits, in turn, are used for purposes of astringent, diuretic, antidiarrheal action and as aphrodisiacs (5). In Bangladesh, pills made from a mixture of leaves and fruits (3 times a day for a week) are used as appetite stimulants, digestive and against weakness (33). Still, a recent study carried out in Brazil mentioned the usefulness of the leaves for the treatment of cough and flu and abdominal pain (32). Other ethnomedicinal studies report the use of the leaves for skin problems, antipyretic activity, diuretic action, treatment of pain, gastrointestinal and respiratory disorders, hepatic disorders, treatment of gonorrhoea and snake bites (5). The veterinary ethnomedicinal use of the species in Trinidad and Tobago is also reported, specifically for the treatment of demodetic mange and other skin parasitism in dogs (6).

Phytoconstituents of Bixa orellana

The chemical components of *B. orellana* can vary according to the part of the plant, location area of the species or even according to edaphic, climatic and environmental factors, in general. The biosynthetic routes of primary and secondary metabolites of plants can be altered by adaptation needs and physiological development (34). Considering all the plant organs of *B. orellana*, the main compounds found are the pigments of the carotenoid class, which includes bixin, as well as some terpenoids, tocotrienols and flavonoids (luteolin and apigenin, mainly) (35, 36). *B. orellana* has undergone phytochemical screening which has resulted in the isolation

Table 1. Traditional uses of *B. orellana* in different countries

Country	Uses	Parts used	Sources
Brazil	Body pain, Antipyretic, laxatives, malaria, Antipyretic, laxatives, Insect repellent Condiment, Food colouring	Seeds	(15)
Colombia	Antivenin for snakebites	Seeds	(16)
	Gonorrhea, Aphrodisiac	Leaves	
Guatemala	Gonorrhea, dysentery, Hepatitis Blood diseases	Leaves	(16)
	Diabetes	Roots	(17)
Honduras	Aromatic, food coloring,	Seeds	(18)
	Pain, digestive, dysentery	Leaves	(19)
	Diabetes	Seeds	(19)
Jamaica	Antipyretic, skin problems	Leaves	(20)
	Alcoholic hepatitis, worms	Roots	(20)
	Antipyretic aphrodisiac, dysentery, astringent, stomach	Seeds	(21)
Argentina	Antipyretic, cardiotonic, antidiarrheal	Seeds	(22)
	Antidiarrheal, dyes, condiment	Seeds	(23)
Nicaragua	Respiratory and pulmonary disorders, diarrhea diuretic,burns	Leaves + Seeds	(24)
	Labor pains	Seeds	
	Cough, cold, diuretic, diarrhea, burns, labor pains	Seeds	(24)
Paraguay	Insecticide, repellent	Seeds	(25)
	Diabetes	Seeds	(26)
Peru	Aphrodisiac, aphrodisiac, diuretic, Antidisenteria, Astringent	Fruits	(27)
	Antipyretic, skin problems	Leaves	(20)
	Alcoholic hepatitis, worms	Roots	(20)
Trinidad and Tobago	Antipyretic, aphrodisiac, dysentery, astringen, stomach	Seeds	(27)
	Diuretic	Leaves	(28)
	Diabetes	Roots	(29)

and identification of several chemical compounds with various structural characteristics. Carotenoids, apocarotenoids, sterols, aliphatic compounds, monoterpenes, sesquiterpenes, triterpenoids and other chemical elements have all been found and isolated, primarily from the seeds, seed coat and leaves of *B. orellana.*

The phytochemical screening of the crude aqueous extract of *B. orellana* indicates the presence of flavonoids, tannins, anthraquinones, saponins and terpenoids. Acetone extract indicates the presence of terpenoids and glycosides. Methanol extract indicates the presence of tannins and glycosides. Ethanolic extract indicates the presence of tannins, flavonoids, saponins, steroids and terpenoids. Hexane extract indicates the presence of glycosides. Terpenoids can be isolated in ether extract. Ethyl acetate extract indicates the presence of tannins, flavonoids, saponins, steroids and terpenoids. The Hydroethanolic extract of *B. orellana* (leaves) showed the presence of terpenes, flavonoids, tannins, coumarins and saponins and absence of alkaloids and anthraquinones (37).

Other Chemical components

Bixin, a carotenoid that appears red, is the primary pigment found in high concentrations in the aril of annatto seeds. This pigment is chiefly responsible for the seeds' dyeing properties, with its levels reaching up to 5.0 % in some cases. However, in other seeds, the concentration can be below 2.0 %. Given that the commercial value of the seeds depends on their bixin content, a concentration of more than 2.5 % is generally needed for export purposes. Bixin was first extracted from the seeds of *B. orellana* in 1875. Its full chemical structure and stereochemistry were later elucidated in 1961 using 1H and 13C-NMR techniques. Bixin is part of a rare group of natural apocarotenoids, which are produced through the oxidative breakdown of C40 carotenoids. Bixin is composed of a chain of 25 carbon atoms and has the molecular formula $C_{25}H_{30}O_4$, with a molecular weight of 394.51. The structure includes a carboxylic acid group at one end and a methyl ester group at the other. In nature, bixin typically exists as the 16-Z (cis) isomer. However, during extraction, it can isomerize to the 16-E (trans) form, known as isobixin. The primary oily component of annatto seeds is geranylgeraniol, accounting for 1 % of the dry seed weight. Norbixin is a demethylated derivative of bixin (Fig. 2). Although norbixin occurs naturally, it is usually referred to as a saponification product of bixin and is the form used for commercial purposes (38). Over two dozen compounds have been isolated from the seeds of *B. orellana*. In addition to bixin and norbixin, these include isobixin, beta-carotene, cryptoxanthin, lutein, zeaxanthin, orellin, bixin, bixol, crocetin, ishwarane, ellagic acid, salicylic acid, threonine, tomentosic acid, tryptophan and phenylalanine. The seeds also contain 40 to 45 % cellulose, 3.5 to 5.5 % sugars, 0.3 to 0.9 % essential oils, 3 % fixed oils, 1.0 to 4.5 % pigments, 13 to 16 % proteins, as well as alpha and beta-carotene, tannins and saponins (39).

Pharmacology Approaches of B. Orellana

Due to the many ethnomedical benefits of *B. orellana* during the past few decades, numerous pharmacological studies have been started by researchers worldwide. Through scrutiny of its ethnomedical uses, a wide range of biological activities, including 'antibacterial, antifungal, antioxidant, anti-inflammatory, anti-carcinogenic, enhanced gastrointestinal motility, neuropharmacological, anticonvulsant and anti hypercholesterolemic activities have been identified (Table 2) (41-65).

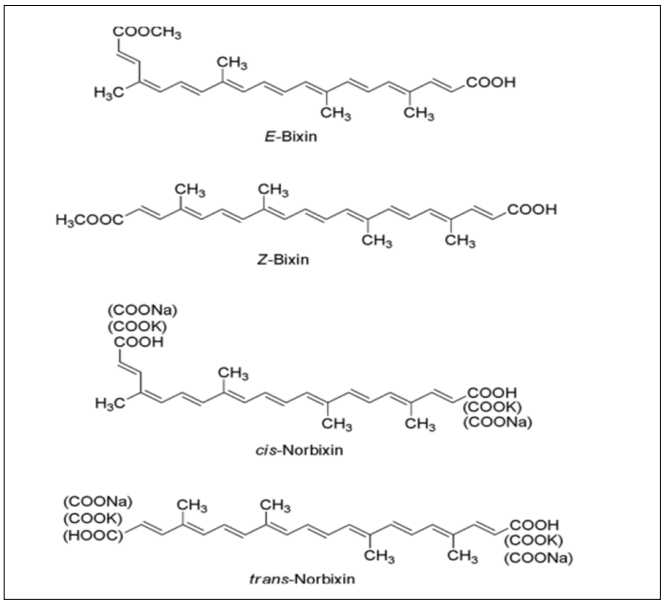

Fig. 2. Chemical structure of Bixin and Norbixin (40).

Antibacterial activity

The antibacterial effectiveness of deseeded and leaf extracts of *B. orellana* were analyzed against both Gram-positive and Gram-negative microorganisms. The disc diffusion method was used to investigate the antibacterial activity of the *B. orellana* extract agains *Escherichia coli, Staphylococcus aureus, Bacillus cereus and Pseudomonas aeruginosa* using gentamycin sulphate as standard. The results revealed that the antibacterial activity of leaves was more prominent and fruit extracts displayed the same properties at significantly greater concentrations. In *Staphylococcus aureus, Bacillus cereus and Pseudomonas aeruginosa,* the growth of inhibition occurs only in the DMSO (Dimethyl sulphoxide) extract of seeds. The crude ethanolic leaves extract of *B. orellana* has shown antibacterial potential against *S. aureus* with a minimal inhibitory concentration of 62.5-g /mL. Similarly, the ethanolic extract of Bixa seeds also proved to be more active against *E. coli* and *B. cereus* (66).

Antioxidant activity

The simplest way to control oxidation is to employ synthetic antioxidants. They increase the nutritional value and sensory quality of foods while extending their shelf life and reducing the production of undesirable oxidation products. One of the main factors contributing to food decomposition is the antioxidant of lipids and proteins. The antioxidant capacity of B. orellana leaf solvent extracts was evaluated. Significant antioxidant activity was seen in acetone, methanol, chloroform and ether extracts. Thiobarbituric acid reactive substance (TBARS) and peroxide value (POV) were examined over 14-days of refrigeration to assess the antioxidant effects of annatto seeds on pork patties (23,36,67).

Table 2. Biological activities of extracts of *Bixa orellana*

Country	Biological Activity	Plant Parts Used	Organism Tested	Activity	Reference
Argentina	Antibacterial	Leaf extract	*Bacillus subtilis*	Inactive	(41)
			Escherichia coli	Inactive	(41)
			Micrococcus luteus	Inactive	(41)
	Antifungal	Leaf extract	*Pseudomonas aeruginosa*	Inactive	(41)
			Staphylococcus aureus	Inactive	(41)
	Antiviral	Seed	*Aspergillus niger*	Inactive	(41)
			Candida albicans	Inactive	(41)
	Insecticidal	Aerial part	Virus - *Herpes simplex*		(42)
Brazil	Antimalarial	Seed	Mouse	Inactive	(15)
	Antioxidant	Seed	Invitro	Active Active	(11)
	Insect repellent	Seed	Hamster	Active	(25)
	Molluscicidal	Seed	Mosquito	Inactive	(11)
	Mutagenic Toxicity	Seed	Conch	Inactive	(43)
	Antileishmanial	Leaf/Root Seed	Mouse Rat	Inactive	(44)
	Antileishmanial	Seed	*Leishmania amazonensis Leishmania amazonensis*	Active Active	(45)
	Hyperlipidemia		Mouse	Active	(46)
					(47)
					(48)
Colombia	Snake bite	Leaf	Mouse	Active	(12)
Costa Rica	Anti-inflammatory	Root	Rat	Inactive	(49)
Cuba	Positive inotropic effect	Apical part	Guinea pig	Inactive	(50)
	Antimalarial	Seed	*Plasmodium gallinaceum/falciparum*	Active	(51)
	Cytotoxic	Seed	Tumor cells	Active	(52)
Ecuador	Antifungal	Leaf	*Aspergillus niger*	Inactive	(53)
			Candida albicans	Inactive	(53)
			Cryptococcus neoformans	Inactive	(53)
			Fusarium oxysporum	Inactive	(53)
			Neurospora crassa	Inactive	(53)
			Penicillium purpurogenum	Inactive	(53)
			Trichophyton mentagrophytes	Active	(53)
Guatemala	Antibacterial	Leaf	*Escherichia coli*	Inactive	(54)
Guatemala	Antifungal	Leaf	*Pseudomonas aeruginosa*	Inactive	(54)
		Leaf	*Salmonella typhi*	Inactive	(55)
	Antigonorrheal	Leaf	*Shigella dysenteriae Staphylococcus aureus Aspergillus flavus*	Inactive	(55)
	Antitrypanosomal Cytotoxic	Leaf	*Candida albicans*	Inactive	(55)
	Inhibitation of platelet aggregation	Seed	*Microsporum gypseum Neisseria gonorrhea Trypanosoma cruzi Crustacean*	Inactive	(55)
				Active	(56)
				Inactive	(54)
				Inactive	(57)
Hawaii	Contraceptive	Root	*Mouse*	Inactive	(58)
Jamaica	Hypoglycemic Antioxidant	Seed	*Dog*	Active Active	(26)
		Seed	*In vitro*		(40)
Mexico	Allergenic	Seed	*Human*	Active	(59)
Puerto Rica	Molluscicidal	Total plant	*Conch*	Inactive	(60)
Dominican Republic	Cytotoxic	Seed	*Cell culture*	Inactive	(61)
Trinidad and Tobago	Antibacterial	Seed	*Escherichia coli*	Inactive	(62)
			Pseudomonas aeruginosa		
USA	Anticonvulsant	Root	*Mouse*	Inactive	(63)
	Antimalarial	Seed	*Rat*	Inactive	(63)
			Plasmodium gallinaceum	Inactive	(64)
			Salmonella typhimurium Rat	Inactive	(65)
	Mutagenic	Root	*Mouse*	Active	(65)
	Hypotensive Toxicity	Root		Inactive	(64)

Anticancer activity

Due to its high propensity to spread and substantial resistance to the standard therapeutic regimen, cutaneous melanoma is challenging to treat. *In vitro* anticancer potential of the apocarotenoid, bixin was demonstrated using HL60 (leukemia), B16 (melanoma), U20S (osteosarcoma), PC3 (prostate), HCT-116 (colon), MCF-& (breast), DRO (anaplastic thyroid) and BHP- 16 (papillary thyroid) cell lines using dacarbazine as a standard. UPLC- DAD-MS/MS analyses of bioactive extracts from *B. orellana* seeds leads to identification of two apocarotenoids. Bixin was evaluated on A2058 cells expressing the oncogenic BRFA VE600 mutation and resistant to dacarbazine treatment. Bixin have anticancer activity in cultured Hep3B human liver cancer cells via., a combination multiple actions including arrest of cell cycle and inhibition of cell growth and induction of apoptotic cells death through extrinsic and intrinsic pathway and inhibit COX-1 and COX-2 enzymes, growth inhibition against breast, colon stomach, CNS and lungs tumour cells (68, 69).

Anti-inflammatory activity

The anti-inflammatory effect of bixin was caused by the activation of the antioxidant Nrf2 transcription factor, which is effective in accelerating wound healing and minimizing the amount of scar tissue. In the first and second hours following theadministration of carrageenan, oral therapy with bixin encourages a considerable decrease in paw oedema, because bixin can stop neutrophils from migrating to an inflamed location. Orally administered aqueous extract of *B. orellana* exhibits significant anti-inflammatory properties. It was demonstrated that the aqueous extract prevented the paw oedema in rats at oral doses of 50 and 150 mg/kg 30 min after induction. The aqueous extract of the plant has considerable anti considerable anti-inflammatory activity against the acute phase of inflammation, which may be brought on by anti-bradykin in activity. These findings confirm the historical application of *B. orellana* leaves to inflammation. One of the key factors contributingto CNS dysfunction in multiple sclerosis is oxidative stress. Additionally, ROS are the primary oxidative stress mediators and TXNIP/NLRP3 inflammasome initiators. In experimental autoimmune encephalomyelitis mice, bixin suppresses the TXNIP/NLRP3 inflammasome and promotes the NRF2 signaling pathway (70, 71).

Antiosteoporosis activity

Annatto to cotrienol can halt the degenerative changes to the bones in rats receiving buserelin. Three features of healthy bones bone microstructure, calcium content and biological strength have been shown in annatto tocotrienol. bone microstructure, bone calcium content and bone biomechanical properties in a maleosteoporosis model brought on by the GnRH agonist buserelin. An orchiectomy is conducted, which results in reduced testosterone production, to artificially induceosteoporosis in rate. After orchiectomy, rats lose their androgens and develop a state resembling the illness (72).

Antidiarrheal activity

Urinary incontinence and faecal urgency are symptoms of diarrhoea, which are caused by an imbalance between the mechanisms of intestinal production and absorption. The imbalance frequently occurs with intestinal hypermotility which results in an excessive loss of bodily fluid and electrolytes in the stool. It is associated with viral diseases, food poisoning and other gastrointestinal disorders that are marked by an increase in bowel frequency. The methanolic extract of *B. orellana* causes maximal antidiarrheal activity (85 % at 500 mg/kg). Hydroethanolic extract of antidiarrheal compounds such as flavonoids, tannins, terpenes, saponins and sterols which are known for their antidiarrheal activity (73).

Antimalarial activity

The Potential chemical constituents of *B. orellana* which is present in the hairy root line was used for the treatment of malaria. The anti-malarial activity of B. orellana against malaria strains 3D7 and K1. The root line was cultured in a modified liquid Murashige and Skoog medium (MSV). The results revealed that the root line of B. orellana showed significant anti-malarial activity against the malarial strains. The hairy roots show that anti-malarial property in the 15-20 mm range and no cytotoxicity was observed in mammalian cell line (74, 75).

Conclusion

Bixa orellana holds immense industrial, medicinal and cultural significance. Its natural pigments, bixin and norbixin, offer safer alternatives to synthetic colorants, widely used in food and beverages with FDA approval. Medicinally, it exhibits antimicrobial, anticancer, antidiabetic and antimalarial properties, showcasing its therapeutic potential. While research highlights its vast biological and pharmacological activities, further studies are essential to uncover the precise roles of its compounds. As exploration continues, *B. orellana's* value across industries will undoubtedly expand, affirming its status as a versatile and impactful natural resource.

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Authors' contributions

PSD drafted the manuscript. PR, PK, BS, MS and MK participated in the collection of review materials. NR, SU, PM, GA, MM and RR participated in the discussion and designing the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Raddatz-Mota D, Pérez-Flores LJ, Carrari F, Mendoza-Espinoza JA, de León-Sánchez FD, Pinzón-López LL, et al. Achiote (*Bixa orellana* L.): a natural source of pigment and vitamin E. Journal of Food Science and Technology. 2017;54:1729-41. https://doi.org/10.1007/s13197-017-2579-7
2. Purushothaman T, Irfanamol K. Ethnobotany, Phytochemistry and Pharmacological Efficacy of *Bixa orellana*: A Review. 2021. https://doi.org/10.34293/ sijash.v9i2.4131
3. Smith J. Annatto extracts-chemical and technical assessment. Chem Tech Assess Manual. 2006:1-21.
4. Raju SK, Chandrasekar S, Vengadhajalapathy P, Sundaram R, Periyasamy S, Chinnaraj T, et al. Review on phytochemical composition and pharmacological activities of *Bixa orellana* L. Journal of Pharmaceutical and Biological Sciences. 2022;10(2):57-67. https://doi.org/10.18231/j.jpbs.2022.012
5. Vilar DD, Vilar MS, Moura TF, Raffin FN, Oliveira MR, Franco CF, et al. Traditional uses, chemical constituents and biological activities of *Bixa orellana* L.: a review. The Scientific World Journal. 2014;857292. https://doi.org/10.1155/2014/857292
6. Lans C, Harper T, Georges K, Bridgewater E. Medicinal plants used for dogs in Trinidad and Tobago. Preventive Veterinary Medicine. 2000;45(3-4):201-20. https://doi.org/10.1016/S0167-5877(00)00123-9
7. Silva SN, Amaral CL, Rebouças TN, Morais O. Adoption of conservation practices on farm and selection of varieties by producers of annatto in the city of Vitoria da Conquista-BA. Revista Brasileira de Agroecologica. 2010;5:106-13.
8. Hirko B, Getu A. *Bixa orellana* (Annatto Bixa) A review on use, structure, extraction methods and analysis. Journal of Agronomy, Technology and Engineering Management. 2022;5(1):687-96.
9. Ribeiro JA, Oliveira DT, Passos ML, Barrozo MA. The use of nonlinearity measures to discriminate the equilibrium moisture equations for *Bixa orellana* seeds. Journal of Food Engineering. 2005;66(1):63-8. https://doi.org/10.1016/j.jfoodeng.2004.02.040
10. Otero R, Fonnegra R, Jiménez SL, Núñez V, Evans N, Alzate SP, et al. Snakebites and ethnobotany in the northwest region of Colombia: Part I: traditional use of plants. Journal of Ethnopharmacology. 2000;71(3):493-504. https://doi.org/10.1016/S0378-8741(00)00243-9
11. Giorgi A, De Marinis P, Granelli G, Chiesa LM, Panseri S. Secondary metabolite profile, antioxidant capacity and mosquito repellent activity of *Bixa orellana* from Brazilian Amazon region. Journal of Chemistry. 2013;409826. https://doi.org/10.1155/2013/409826
12. Otero R, Núñez V, Barona J, Fonnegra R, Jiménez SL, Osorio RG, et al. Snakebites and ethnobotany in the northwest region of Colombia: Part III: Neutralization of the haemorrhagic effect of Bothrops atrox venom. Journal of Ethnopharmacology. 2000;73(1-2):233-41. https://doi.org/10.1016/S0378-8741(00)00321-4
13. Yong YK, Zakaria ZA, Kadir AA, Somchit MN, Ee Cheng Lian G, Ahmad Z. Chemical constituents and antihistamine activity of *Bixa orellana* leaf extract. BMC Complementary and Alternative Medicine. 2013;13:1-7. https://doi.org/10.1186/1472-6882-13-32
14. Leal F, Clavijo CM. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. Revista Unellez de Ciencia Tecnologia., 28:78–86. 2010.
15. Brandão MG, Grandi TS, Rocha EM, Sawyer DR, Krettli AU. Survey of medicinal plants used as antimalarials in the Amazon. Journal of Ethnopharmacology. 1992;36(2):175-82. https://doi.org/10.1016/0378-8741(92)90018-M
16. Akshatha V, Giridhar P, Ravishankar GA. Food, ethanobotanical and diversified applications of *Bixa orellana* L.: a scope for its improvement through biotechnological mediation. Indian Journal of Fundamental and Applied Life Sciences. 2011;1(4):9-31. http://ir.cftri.res.in/id/eprint/10913
17. Girón LM, Freire V, Alonzo A, Cáceres A. Ethnobotanical survey of the medicinal flora used by the Caribs of Guatemala. Journal of Ethnopharmacology. 1991;34(2-3):173-87. https://doi.org/10.1016/0378-8741(91)90035-C
18. Lentz DL. Medicinal and other economic plants of the Paya of Honduras. Economic botany. 1993:358-70. https://www.jstor.org/stable/4255543
19. Lentz DL, Clark AM, Hufford CD, Meurer-Grimes B, Passreiter CM, Cordero J, et al. Antimicrobial properties of Honduran medicinal plants. Journal of Ethnopharmacology. 1998;63(3):253-63. https://doi.org/10.1016/S0378-8741(98)00100-7
20. Duke JA. Amazonian ethnobotanical dictionary. CRC press; 2018.
21. Ramirez VR, Mostacero LJ, Garcia AE. Vegetable employed in traditional medicine. J Univ Trujillo. 1988;1:54-8.
22. Bandoni AL, Mendiondo ME, Rondina RV, Coussio JD. Survey of Argentine medicinal plants. I. Folklore and Phytochemical Screening. 1972.
23. Garcia GH, Campos R, Torres RD, Broussalis A, Ferraro G, Martino V, et al. Antiherpetic activity of some Argentine medicinal plants. 1990.
24. Coe FG, Anderson GJ. Screening of medicinal plants used by the Garifuna of Eastern Nicaragua for bioactive compounds. Journal of Ethnopharmacology. 1996;53(1):29-50. https://doi.org/10.1016/0378-8741(96)01424-9
25. de Arias AR, SchmedaÆHirschmann G, Falcao A. Feeding deterrency and insecticidal effects of plant extracts on Lutzomyia longipalpis. Phytotherapy Research. 1992;6(2):64-7. https://doi.org/10.1002/ptr.2650060203
26. Morrison EY, West ME. The effect of *Bixa orellana* (Annatto) on blood sugar levels in the anaesthetized dog. West Indian med. J. 1985:38-42.
27. Ramirez VR, Mostacero LJ, Garcia AE. Vegetable employed in traditional medicine. J Univ Trujillo. 1988;1:54-8.
28. Simpson GE. Folk medicine in Trinidad. The Journal of American Folklore. 1962;75(298):326-40. https://doi.org/10.2307/538368
29. Mahabir D, Gulliford MC. Use of medicinal plants for diabetes in Trinidad and Tobago. Revista Panamericana de Salud Pública. 1997;1:174-9. https://doi.org/10.1590/S1020-49891997000300002
30. Nataru S, Pulicherla Y, Gaddala B. A review on medicinal plants as a potential source for cancer. Int J Pharm Sci Rev Res. 2014;26(1):235-48.
31. Revilla J. Plantas da Amazônia: oportunidades econômicas e sustentáveis. Sebrae; 2001.
32. Manganelli L, Fonseca YS, Ledo NA, Barbosa LM, Borges GF, Ramos GA, et al. Estudo etnobotÃ¢nico do uso de *Bixa orellana* L. (urucum) por agricultores do Extremo Sul da Bahia. Revista Cubana de Plantas Medicinales. 2018;23(3).
33. Rahmatullah M, Noman A, Hossan MS, Rashid MH, Rahman T, Chowdhury MH, et al. A survey of medicinal plants in two areas of Dinajpur district, Bangladesh including plants which can be used as functional foods. American Eurasian Journal of Sustainable Agriculture. 2009;3(4):862-76.
34. Akula Ramakrishna AR, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in plants. 2011. https://doi.org/10.4161/psb.6.11.17613

35. Radhika B, Begum N, Srisailam K, Reddy VM. Diuretic activity of *Bixa orellana* Linn. leaf extracts. 2010.

36. Ramírez T. Evaluación de la Actividad Hipoglicemiante del Extracto Acuoso de las Hojas de *Bixa orellana* "Achiote"[Tesis]. Ayacucho (Perú): Universidad Nacional de San Cristóbal de Huamanga. 2001:23-6.

37. Cárdenas-Conejo Y, Carballo-Uicab V, Lieberman M, Aguilar-Espinosa M, Comai L, Rivera-Madrid R. De novo transcriptome sequencing in *Bixa orellana* to identify genes involved in methylerythritol phosphate, carotenoid and bixin biosynthesis. BMC genomics. 2015;16:1-9. https://doi.org/10.1186/s12864-015-2065-4

38. Beretta G, Gelmini F, Fontana F, Moretti RM, Montagnani Marelli M, Limonta P. Semi-preparative HPLC purification of δ-tocotrienol (δ-T3) from *Elaeis guineensis* Jacq. and *Bixa orellana* L. and evaluation of its *in vitro* anticancer activity in human A375 melanoma cells. Natural product research. 2018;32(10):1130-5. https://doi.org/10.1080/14786419.2017.1320793

39. Taylor, L. Technical data report for bitter melon (*Momordica charantia*). Herbal secrets of the rainforest.12002:103-114.

40. Abayomi M, Adebayo AS, Bennett D, Porter R, Shelly-Campbell J. *In vitro* antioxidant activity of *Bixa orellana* (Annatto) seed extract. Journal of Applied Pharmaceutical Science. 2014;4(2):101-6. https://doi.org/10.7324/JAPS.2014.40216

41. Penna CA, Radice M, Gutkind GO, Baren CV, Broussalis A, Muschietti L, et al. Antibacterial and antifungal activities of some Argentinean plants. 1994.

42. Broussalis AM, Ferraro GE, Martino VS, Pinzón R, Coussio JD, Alvarez JC. Argentine plants as potential source of insecticidal compounds. Journal of Ethnopharmacology. 1999;67(2):219-23. https://doi.org/10.1016/S0378-8741(98)00216-5

43. Sousa MD, Rouquayrol MZ. Molluscicidal activity of plants from north-east Brazil. 1974.

44. de Lima RA, Azevedo L, Ribeiro LR, Salvadori DM. Study on the mutagenicity and antimutagenicity of a natural food colour (annatto) in mouse bone marrow cells. Food and Chemical Toxicology. 2003;41(2):189-92. https://doi.org/10.1016/S0278-6915(02)00208-9

45. Hagiwara A, Imai N, Ichihara T, Sano M, Tamano S, Aoki H, et al. A thirteen-week oral toxicity study of annatto extract (norbixin), a natural food color extracted from the seed coat of annatto (*Bixa orellana* L.), in Sprague–Dawley rats. Food and Chemical Toxicology. 2003;41(8):1157-64. https://doi.org/10.1016/S0278-6915(03)00104-2

46. Rodrigues CA, Silva RB, Marqués MJ, Chavasco JK. Evaluation of antiparasitic activity of hydroethanolic extracts from root, stem and leaf of *Bixa orellana* L. on Leishmania amazonensis samples. Revista da Universidade Vale do Rio Verde. 2013;10(2):384-91. http://doi.org/10.5892/ruvrv.2012.102.384391

47. Lopes MV, Desoti VC, Caleare AD, Ueda-Nakamura T, Silva SO, Nakamura CV. Mitochondria superoxide anion production contributes to geranylgeraniol-induced death in *Leishmania amazonensis*. Evidence-Based Complementary and Alternative Medicine. 2012;2012(1):298320. https://doi.org/10.1155/2012/298320

48. Ferreira JM, Sousa DF, Dantas MB, Fonseca SG, Menezes DB, Martins AM, et al. Effects of *Bixa orellana* L. seeds on hyperlipidemia. Phytotherapy Research. 2013;27(1):144-7. https://doi.org/10.1002/ptr.4675

49. Benoit PS, Fong HH, Svoboda GH, Farmsworth NR. Biological and phytochemical evaluation of plants. XIV. Anti-inflammatory evaluation of 163 species of plants. Lloydia. 1976;39(2-3):160-71.

50. Carbajal D, Casaco A, Arruzazabala L, Gonzalez R, Fuentes V. Pharmacological screening of plant decoctions commonly used in Cuban folk medicine. Journal of Ethnopharmacology. 1991;33(1-2):21-4. https://doi.org/10.1016/0378-8741(91)90155-7

51. Fernández-Calienes Valdés A, Mendiola Martínez J, Acuña Rodríguez D, Scull Lizama R, Gutiérrez Gaitén Y. Actividad antimalárica de un extracto hidroalcohólico de *Bixa orellana* L. Revista Cubana de Medicina Tropical. 2011;63(2):181-5.

52. Garcia AD, S´anchez HR, RS Lizama. "Citotoxicidadde extractos de plantasmedicinalessobre la l´ıneacellular de carcinoma de pulm´onhumano A549." Revista Cubana deFarm´acia. 2011.45:101–108.

53. Freixa B, Vila R, Vargas L, Lozano N, Adzet T, Cañigueral S. Screening for antifungal activity of nineteen Latin American plants. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 1998;12(6):427-30. https://doi.org/10.1002/(SICI)1099-1573(199809)12:6<427::AID-PTR338>3.0.CO;2-X

54. Cáceres A, López B, González S, Berger I, Tada I, et al. Plants used in Guatemala for the treatment of protozoal infections. I. Screening of activity to bacteria, fungi and American trypanosomes of 13 native plants. Journal of Ethnopharmacology. 1998;62(3):195-202. https://doi.org/10.1016/S0378-8741(98)00140-8

55. Caceres A, Cano O, Samayoa B, Aguilar L. Plants used in Guatemala for the treatment of gastrointestinal disorders. 1. Screening of 84 plants against enterobacteria. Journal of Ethnopharmacology. 1990;30(1):55-73. https://doi.org/10.1016/0378-8741(90)90017-N

56. Caceres A, Menendez H, Mendez E, Cohobón E, Samayoa BE, Jauregui E, et al. Antigonorrhoeal activity of plants used in Guatemala for the treatment of sexually transmitted diseases. Journal of Ethnopharmacology. 1995;48(2):85-8. https://doi.org/10.1016/0378-8741(95)01288-O

57. Villar R, Calleja JM, Morales C, Cáceres A. Screening of 17 Guatemalan medicinal plants for platelet antiaggregant activity. Phytotherapy Research: An International Journal Devoted to Medical and Scientific Research on Plants and Plant Products. 1997;11(6):441-5. https://doi.org/10.1002/(SICI)1099-1573(199709)11:6<441::AID-PTR126>3.0.CO;2-T

58. Matsui AD, Hoskin S, Kashiwagi M, Aguda BW, Zegart BE, Norton TR, et al. A survey of natural products from Hawaii and other areas of the Pacific for an antifertility effect in mice. 1971.

59. Dunham NW, Allard KR. A preliminary pharmacologic investigation of the roots of *Bixa orellana*. Journal of the American Pharmaceutical Association. 1960;49(4):218-9. https://doi.org/10.1002/jps.3030490409

60. Medina FR, Woodbury R. Terrestrial plants molluscicidal to lymnaeid hosts of fascioliasis hepatica in Puerto Rico. 1979.

61. Weniger B, Jiang Y, Oulad-Ali A, Italiano L, Beck JP, Anton R. Biological effects of bixin and *Bixa orellana* extracts on lymphoid cells in culture. Planta Medica. 1993;59(S 1):A680.

62. Chariandy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BP. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. Journal of ethnopharmacology. 1999;64(3):265-70. https://doi.org/10.1016/S0378-8741(98)00130-5

63. Paumgartten FJ, De-Carvalho RR, Araujo IB, Pinto FM, Borges OO, et al. Evaluation of the developmental toxicity of annatto in the rat. Food and Chemical Toxicology. 2002;40(11):1595-601. https://doi.org/10.1016/S0278-6915(02)00133-3

64. Spencer CE. Survey of plants for antimalarial activity. 1947.

65. Mikkelsen H, Larsen JC, Tarding F. Hypersensitivity reactions to food colours with special reference to the natural colour annatto extract (butter colour). InToxicological Aspects of Food Safety: Proceedings of the European Society of Toxicology. Meeting held in Copenhagen, June 19–22, 1977 1978 Jan 1 (pp. 141-143). Berlin, Heidelberg: Springer Berlin Heidelberg. https://

doi.org/10.1007/978-3-642-66896-8_16

66. Venugopalan A, Giridhar P. Bacterial growth inhibition potential of annatto plant parts. Asian Pacific Journal of Tropical Biomedicine. 2012;2(3):S1879-82. https://doi.org/10.1016/S2221-1691(12)60513-9

67. Van Cuong T, Chin KB. Effects of annatto (*Bixa orellana* L.) seeds powder on physicochemical properties, antioxidant and antimicrobial activities of pork patties during refrigerated storage. Korean Journal for Food Science of Animal Resources. 2016;36 (4):476. https://doi.org/10.5851/kosfa.2016.36.4.476

68. de Oliveira Júnior RG, Bonnet A, Braconnier E, Groult H, Prunier G, Beaugeard L, et al. Bixin, an apocarotenoid isolated from *Bixa orellana* L., sensitizes human melanoma cells to dacarbazine-induced apoptosis through ROS-mediated cytotoxicity. Food and Chemical Toxicology. 2019; 125:549-61. https://doi.org/10.1016/j.fct.2019.02.013

69. Kumar Y, Phaniendra A, Periyasamy L. Bixin triggers apoptosis of human Hep3B hepatocellular carcinoma cells: an insight to molecular and in silico approach. Nutrition and Cancer. 2018; 70 (6):971-83. https://doi.org/10.1080/01635581.2018.1490445

70. Yoke Keong Y, Arifah AK, Sukardi S, Roslida AH, Somchit MN, Zuraini A. *Bixa orellana* leaves extract inhibits bradykinin-induced inflammation through suppression of nitric oxide production. Medical Principles and Practice. 2011; 20(2):142-6. https://doi.org/10.1159/000319907

71. Pacheco SD, Gasparin AT, Jesus CH, Sotomaior BB, Ventura AC, Redivo DD, et al. Antinociceptive and anti-inflammatory effects of bixin, a carotenoid extracted from the seeds of *Bixa orellana*. Planta Medica. 2019;85(16):1216-24. https://doi.org/10.1055/a-1008-1238

72. Mohamad NV, Ima-Nirwana S, Chin KY. Effect of tocotrienol from *Bixa orellana* (annatto) on bone microstructure, calcium content and biomechanical strength in a model of male osteoporosis induced by buserelin. Drug design, Development and Therapy. 2018;555-64

73. Fokam Tagne MA, Akaou H, Noubissi PA, Foyet Fondjo A, Rékabi Y, et al. Effect of the hydroethanolic extract of *Bixa orellana* Linn (Bixaceae) leaves on castor oil-induced diarrhea in Swiss Albino mice. Gastroenterology Research and Practice. 2019;6963548. https://doi.org/10.1155/2019/6963548

74. Rivera-Madrid R, Aguilar-Espinosa M, Cárdenas-Conejo Y, Garza-Caligaris LE. Carotenoid derivates in achiote (*Bixa orellana*) seeds: synthesis and health promoting properties. Frontiers in Plant Science. 2016;7:1406. https://doi.org/10.3389/fpls.2016.01406

75. Zhai B, Clark J, Ling T, Connelly M, Medina-Bolivar F, Rivas F. Antimalarial evaluation of the chemical constituents of hairy root culture of *Bixa orellana* L. Molecules. 2014;19(1):756-66. https://doi.org/10.3390/molecules19010756