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REVIEW

Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications – A review

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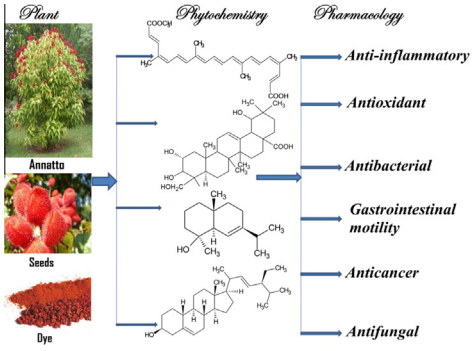
GRAPHICAL ABSTRACT



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ABSTRACT

Bixa orellana commonly known as annatto is one of the oldest known natural dye yielding plants native to Central and South America. Various parts of annatto have been widely used in the traditional medical system for prevention and treatment of a wide number of health disorders. The plethora of traditional uses has encouraged researchers to identify and isolate

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phytochemicals from all parts of this plant. Carotenoids, apocarotenoids, terpenes, terpenoids, sterols, and aliphatic compounds are main compounds found in all parts of this plant and are reported to exhibit a wide range of pharmacological activities. In recent years annatto has received tremendous scientific interest mainly due to the isolation of yellow–orange natural dye from its seeds which exhibits high biodegradability, low toxicity, and compatibility with the environment. Considerable research work has already been done and is currently underway for its applications in food, textile, leather, cosmetic, solar cells, and other industries. The present review provides up-to-date systematic and organized information on the traditional usage, phytochemistry and pharmacology of annatto. It also highlights its non-food industrial applications in order to bring more interest on this dye plant, identifies the existing gaps and provides potential for future studies. Studies reported in this review have demonstrated that annatto holds a great potential for being exploited as source of drugs and a potential natural dye. However, further efforts are required to identify extract biomolecules and their action mechanisms in exhibiting certain biological activities in order to understand the full phytochemical profile and the complex pharmacological effects of this plant.

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Introduction

Bixa orellana L. commonly known as annatto belongs to the family *Bixa*ceae. It is 3–6 m high bush native to Central and South America and is one of the oldest known natural dye yielding plants, Fig. 1. It was named after the Spanish conquistador Francisco de Orellana and has been used earlier for body painting, treatment for heartburn and stomach distress, sunscreen, and repelling insects, and to ward off evil [1]. Annatto has been used for centuries in many parts of the world for the prevention and treatment of a number of heath disorders such as constipation, fevers, heartburn, asthma, scabies, ulcers, diarrhea, stomach upset, skin diseases, measles, anecdotal treatment of diabetes, allergy, leprosy, infectious diseases, burns, measles, gonorrhea, diarrhea, asthma, angina, tumors, skin problems, and urinary infections (oral and topic) [2,3]. The pulp from seeds of this plant has long been used topically by indigenous people to enhance the beauty of lips which has led to the origin of *B. orellana's* nick name as lipstick tree [4]. Annatto has enormous number of applications in coloring and bleaching of dairy food products especially bakery products, cream deserts, butter milk deserts, rice flour, and corn starch [5–7]. Extensive research studies carried out in the last few decades have shown isolation of several different classes of phytoconstituents including carotenoids, apocarotenoids, sterols, aliphatic compounds, monoterpenes and sesquiterpenes, triterpenoids, volatile oils and other miscellaneous compounds from all parts of this plant [8–10]. These phytochemicals exhibit a wide range of pharmacological activities that include antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, enhanced gastrointestinal motility, neuropharmacological, anticonvulsant, analgesic, and antidiarrheal activities [11–15].

Modern investigations on this plant have revealed the presence of natural reddish-yellow dye in seeds of *B. orellana*. The fruit of the *B. orellana* tree consists of 10–50 seeds of the size of grape seeds covered with a thin layer of soft, slightly sticky vermilion pulp [16]. Seeds are characterized by substantial amount of carotenoid compounds mainly apocarotenoid bixin, nor-bixin and other less important cryptoxanthin, lutein, zeaxanthin, and methylbixin [17–19]. Numerous pieces of research have been conducted on *B. orellana* plant over the last few years; however, there is a paucity of comprehensive review

Fig. 1 (a) Plant (b) leaves and flower, and (c) seeds and dye.

articles on this potential natural dye plant [4,6,14]. Keeping in view the tremendous interest in this dye containing plant, we herein summarize up-to-date information on the phytochemistry, and biological activities of annatto. Finally this review also highlights its important industrial applications with critical analysis of the existing gaps and potential for future studies.

Method

An extensive and systematic review of the existing literature was collected from scientific journals, books, reports and worldwide accepted databases (Scopus, ScienceDirect, Scifinder, Medline, Springer, and Google Scholar) using different search key words such as annatto, *B. orellana*, phytochemistry, pharmacology, antibacterial activity and dye.

Phytochemistry

Phytochemical screening of *Bixa orellana* carried out so far has led to the isolation and identification of a number of structurally diverse chemical compounds. There are many chemical constituents including carotenoids, apocarotenoids, sterols, aliphatic compounds, monoterpenes and sesquiterpenes, triterpenoids, and other miscellaneous compounds that have been identified and isolated mostly from seeds, seed coats and leaves of this plant. In this part of the review, we describe the major chemical constituents, their structures and their isolation from different parts of this plant, Table 1.

Carotenoids

The main compounds found in *B. orellana* plant are carotenoids and apocarotenoids. Several phytochemical studies have been performed on isolation and identification of carotenoids and apocarotenoids of various extracts. Most of the carotenoids have been isolated from seed and seed coats. Bixin **(1)** [methylhydrogen-(9′*Z*)-6,6′-diapocarotene-6,6′-dioate] is the major carotenoid compound present in *B. orellana* seed coat and accounts for 80% in addition to the presence of other carotenoids in trace amounts [20,21]. Tirimanna identified and isolated β-carotene, cryptoxanthin, lutein **(2)**, zeaxanthin **(3)**, and methyl bixin **(4)** in addition to bixin and nor-bixin **(5)** from seeds by thin layer chromatography [19]. Chemical investigation of methanol seed extract has resulted in the identification of the apocarotenoids methyl bixin (dimethylhydrogen-(9′*Z*)-6,6′-diapocarotene-6,6′-dioate) **(4)** [22]. In a series of phytochemical investigations Mercadante et al. reported a number of apocarotenoids from *B. orellana* seed coat. In 1996, they successfully isolated methyl-9′*Z*-apo-6′-lycopenoate **(6)** from the seed coats [23].

Methyl-(7*Z*,9*Z*,9′*Z*)-apo-6′-lycopenoate **(7)**, methyl-(9*Z*)-apo-8′-lycopenoate **(8)**, methyl-(*all-E*)-apo-8′-lycopenoate **(9)**, and methyl-(*all-E*)-apo-6′-lycopenoate **(10)** were also isolated from seed coat of *B. orellana* [17]. In 1997, six minor diapocarotenoids and one C_{14}-carotenoid derivative were isolated from the seed coat and were named dimethyl-(9Z,9′Z)-6,6′-dia pocarotene-6,6′-dioate **(4)**, methyl-(9Z)-10′-oxo-6,10′-diapocar oene-6-oate **(11)**, methyl-(9Z)-6′-oxo-6,5′-diapocaroene-6-oate **(12)**, methyl-(9Z)-6′-oxo-6,6′-diapocaroene-6-oate **(13)**, and m ethyl-(4Z)-4,8-dimethyl-12-oxododecyl-2,4,6,8,10-pentaenoate **(14)** [24]. In another study conducted two years later, 6-geranyl geranyl-8′-methyl-6,8′-diapocaroten-6,8′-dioate **(15)**, 6-geranyl geranyl-6′-methyl(9′Z)-6,6′-diapocaroten-6,6′-dioate **(16)** and 6-geranylgeranyl-6′-methyl-6,6′-diapocaroten-6,6′-dioate **(17)** were also successfully obtained from seeds of B. orellana [8]. The chemical structures of isolated carotenoids are shown in Fig. 2.

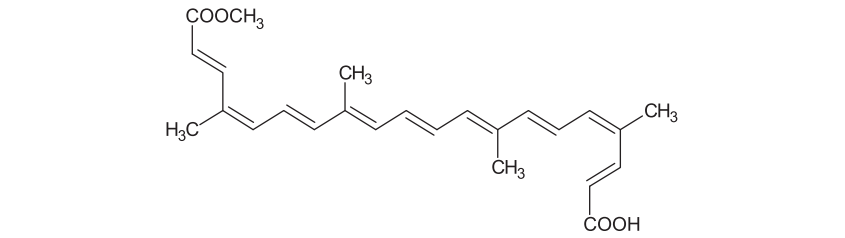
Terpenoids and terpenes

Terpenoids mainly C_{20}-terpene alcohol *all*-geranylgeraniol as a major chemical component in *Bixa orellena* were isolated

Table 1 Chemical constituents of *Bixa orellana*.

S. no.	Classification	Components	Plant part	References
	Carotenoids			
(1)		Methylhydrogen-(9′Z)-6,6′-diapocarotene-6, 6′-dioate (Bixin)	Seed coat	[22]
(2)		Lutein	Seeds	[19]
(3)		Zeaxanthin	Seeds	[19]
(4)		Dimethyl-(9Z,9′Z)-6,6′-diapocarotene-6,6′-dioate	Seed coat	[22,24]
(5)		NorBixin	Seeds	[86]
(6)		Methyl (9′Z)-apo-6′-lycopenoate	Seed coat	[23]
(7)		Methyl-(7Z,9Z,9′Z)-apo-6′-lycopenoate	Seed coat	[23,24]
(8)		Methyl-(9Z)-apo-8′-lycopenoate	Seed coat	[23]
(9)		Methyl-(all-E)-apo-8′-lycopenoate	Seed coat	[23]
(10)		Methyl-(all-E)-apo-6′-lycopenoate	Seed coat	[17]
(11)		Methyl (9*Z*)-10′-oxo-6,10′-diapocaroten-6-oate	Seeds	[24]
(12)		Methyl (9*Z*)-6′-oxo-6,5′-diapocaroten-6-oate	Seeds	[24]
(13)		Methyl (9Z)-6′-oxo-6,6′-dioapocarotene-6-oate	Seeds	[24]
(14)		Methyl-(4Z)-4,8-dimethyl-12-oxododecyl-2,4,6,8,10-pentaenoate	Seeds	[24]
(15)		6-Geranylgeranyl-8′-methyl-6, 8′-diapocaroten-6, 8′-dioate	Seeds	[8]
(16)		6-Geranylgeranyl-6′-methyl (9′*Z*)-6, 6′-diapocaroten-6,6′-dioate	Seeds	[8]
(17)		6-Geranylgeranyl-6′-methyl-6,6′-diapocaroten-6, 6′-dioate	Seeds	[8]
(18)		Trans-bixin	Seeds	[103]
	Terpenoids			
(19)		Farnesylacetone	Seeds	[22]
(20)		Geranylgeranyl octadecanoate	Seeds	[22]
(21)		Geranylgeranyl formate	Seeds	[22]
(22)		δ-Tocotrienol	Seeds	[25]
(23)		β-Tocotrienol	Seeds	[25]
	Terpenes			
(24)		β-Humulene	Roots	[26]
(25)		α-Carpophyllene	Leaves and roots	[12,26]
(26)		α-Copaene	Leaves and roots	[12,26]
(27)		α-Elemene	Leaves	[12]
(28)		Cis-ocimene	Leaves	[12]
(29)		Tomentosic acid	Roots	[104]
	Volatile compounds			
(30)		(Z,E)- farnesyl acetate (11.6%)	Seed oil	[27]
(31)		Occidentalol acetate (9.7%)	Seed oil	[27]
(32)		Spathulenol (9.6%)	Roots, seed oil	[26,27]
(33)		Ishwarane (9.1%)	Seed oil	[27,105]
	Other compounds			
(34)		Acetic acid	Roots	[10]
(35)		2-Butanamine	Roots	[10]
(36)		Pentanoic acid	Roots	[10]
(37)		Phenol	Roots	[10]
(38)		Pantolactone	Roots	[10]
(39)		Benzoic acid	Roots	[10]
(40)		Phytol	Leaves	[37]
(41)		Stigmasterol	Leaves	[37]
(42)		Sitosterol	Leaves	[37]
(43)		Leucocyanidin	Leaves	[28]
(44)		Ellagic acid	Leaves	[28]
(45)		Luteolin	Leaves	[28]
(46)		Apigenin	Leaves	[28]

by Jondiko and Pattenden. Other terpenes that were isolated and characterized for the first time include farnesylacetone **(19)**, geranylgeranyl octadecanoate **(20)**, geranylgeranyl formate **(21)**, δ-tocotrienol **(22)** and β-tocotrienol **(23)** [22]. Frega et al. reported the presence of tocotrienols mainly δ-tocotrienol from lipid fraction of annatto seeds using thin-layer chromatography. Sesquiterpenes are also a major group of volatile compounds found in annatto extracts [25]. In one of the recent studies on annatto β-humulene **(24)** was the major compound present in annatto extract along with its isomer caryophyllene **(25)** which was present in smaller quantities. Several other sesquiterpenes found usually in water-soluble as well as in oil-soluble extracts include α-copaene **(26)**, and α-elemene **(27)** [26]. Figs. 3 and 4 depict the chemical structures for all the isolated terpenes and terpenoids.



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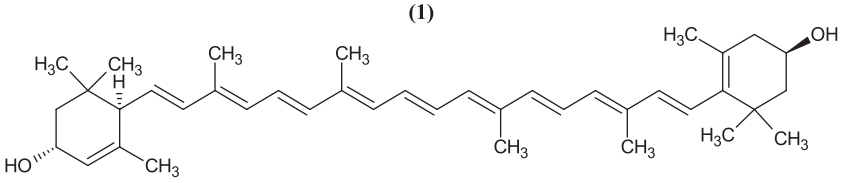

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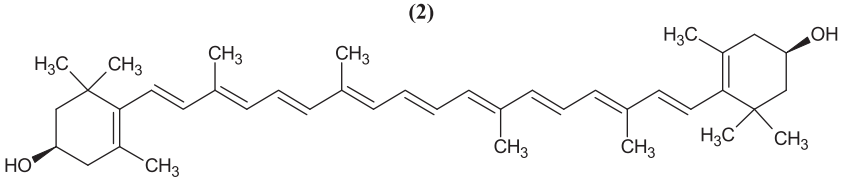

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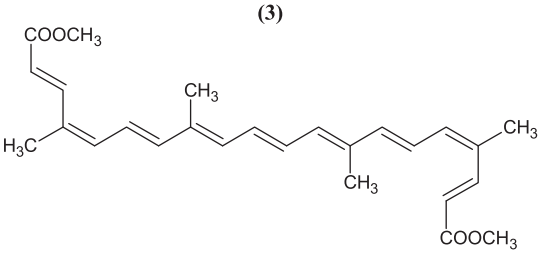

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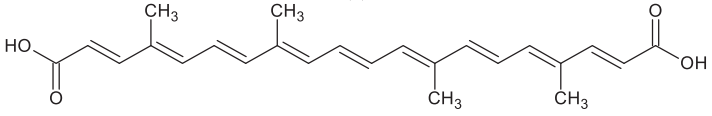

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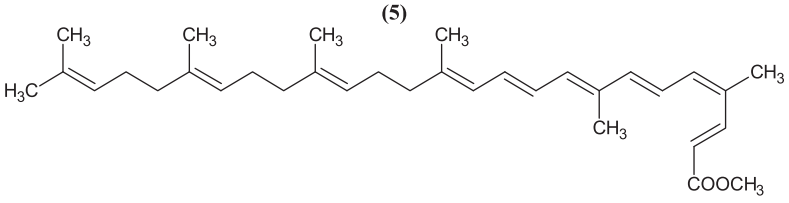

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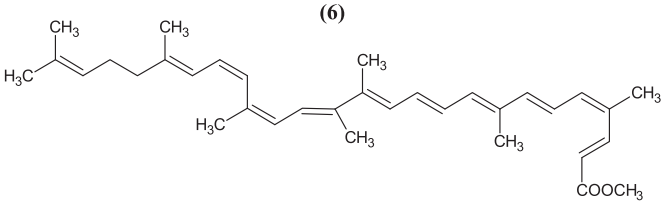

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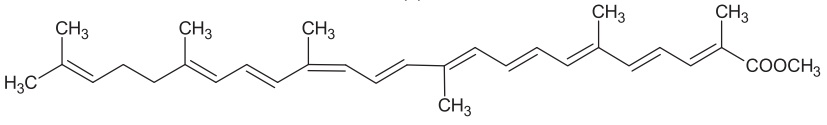

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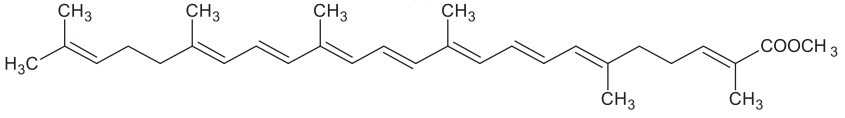

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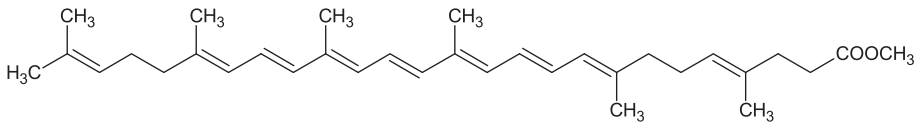
Fig. 2 Chemical structures of carotenoids.



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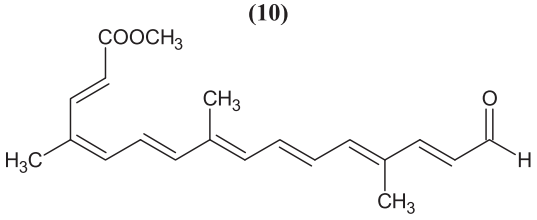

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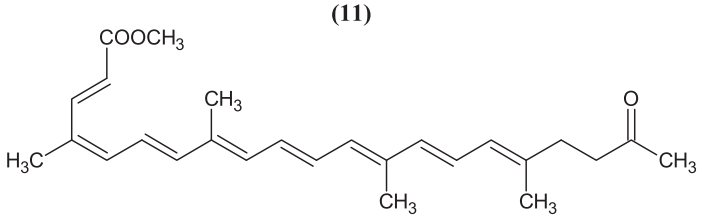

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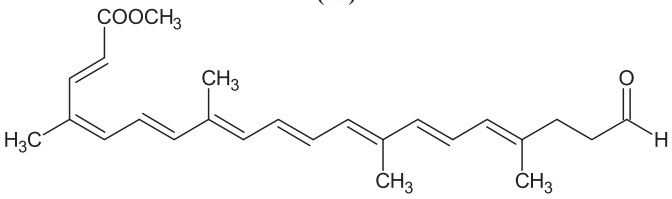

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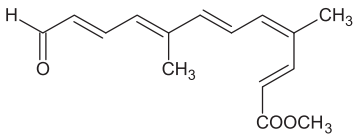

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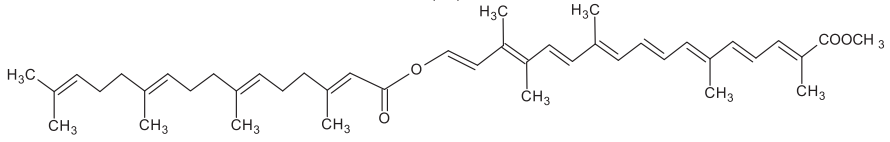

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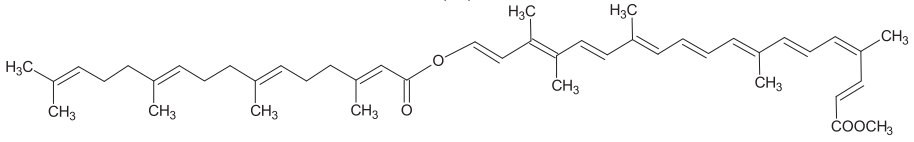

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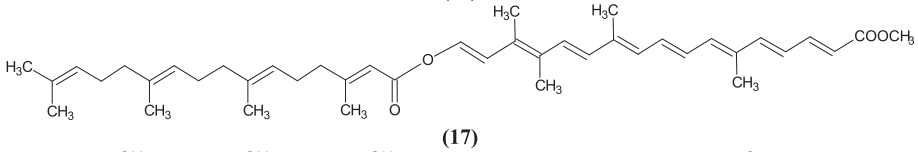

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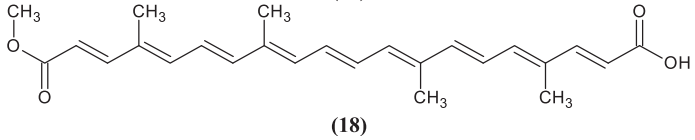

(18)

Fig 2. *(continued)*

Volatile compounds (essential oils)

Table 1 presents a list of volatile compounds isolated from different parts of *B. orellana*. Up to now very few studies have been performed on the extraction and identification of volatile compounds from *Bixa orellana*. One hundred and seven compounds from oil and water soluble annatto extracts were detected by GC/MS in one of the recent studies carried by

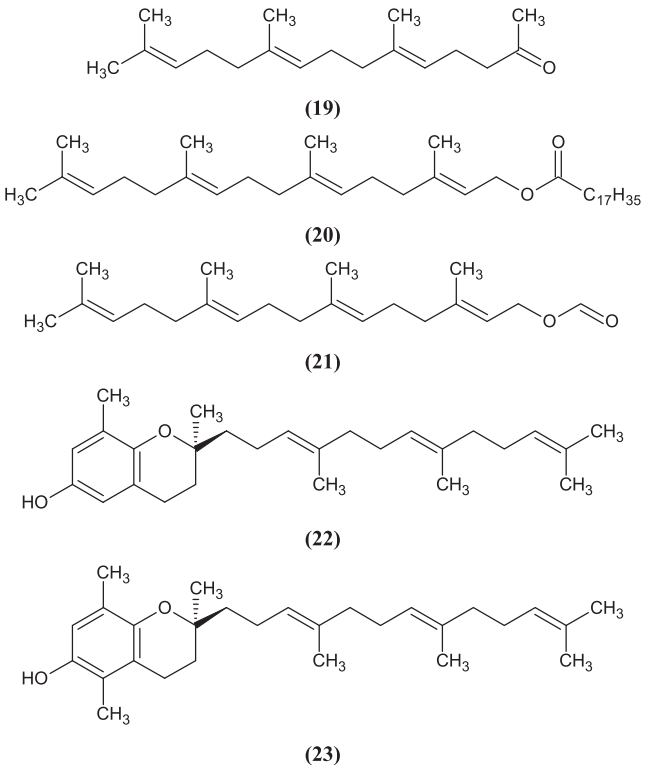

Fig. 3 Chemical structures of terpenoids.

Galindo-Cuspinera et al. using dynamic headspace-solvent desorption technique. The main volatile compounds identified were pentanol and hexanol, 3-hexenol, nonanal, hexanal, and 2-heptenal, dimethylcyclohexane, dimethylhexane and 2-methylheptane, 3-penten-2-one, 3-octanone, 4-methyl-3-penten-2-one, 4-hydroxy-4-methyl-2-pentanone, 6-methyl-5-hepten-2-one, acetic acid, ethyl butyrate, 1,2-propanediol-2-acetate, 3-methylpyridine, *p*-xylene and toluene, δ-elemene, α-pinene, limonene, *β*-myrcene, eucalyptol, *β*-phellandrene, and terpinen-4-ol [26]. Pino and Correa detected thirty-five compounds from seed oil of this plant using GC/MS technique. The major components characterized from seed oil were (Z,E)-farnesyl acetate **(30)** (11.6%), occidentalol acetate **(31)** (9.7%), spathulenol **(32)** (9.6%) and ishwarane **(33)** (9.1%) [27]. Chemical structures are shown in Figs. 5 and 6.

Other miscellaneous compounds

Table 1 lists some other miscellaneous compounds and their chemical structures are given in Fig. 6. GC/MS analysis showed the presence of six major components 2-butanamine **(35)**, acetic acid, pentanoic acid **(36)**, phenol **(37)**, pantolactone **(38)** and benzoic **(39)** [10]. Three new flavone bisulfates have been found in the leaves of *Bixa orellana*. They have been identified as 7-bisulfates of epigenin and luteolin and 8-bisulfate of hypolaetin, confirmed by synthesis [28].

Pharmacodynamics and potential applications

Many pharmacological investigations have been initiated by researchers all over the globe over the past few decades due to varied ethnomedical uses of *B. orellana*. A wide range of biological activities has been described in the literature including antibacterial and antifungal activities, antioxidant and free radical scavenging activities, anti-inflammatory activity, anticarcinogenic activity, enhanced gastrointestinal motility, and neuropharmacological and anticonvulsant activities through detailed observation with respect to its ethnomedical uses. An overview of pharmacological and therapeutic profile of *B. orellana* is described below in detail and briefly summarized in Table 2.

Antibacterial and antifungal activities

Inhibitory actions of the methanol leaf and seed extracts were tested against bacterial and fungal strains. Leaf (MIC = 1000 μg/ml) extracts were more effective and possessed antimicrobial activity against a wide variety of bacteria and fungi, showing greatest activity against *Salmonella typhi* (MIC = 31.25 μg/mL) and *Acinetobacter*

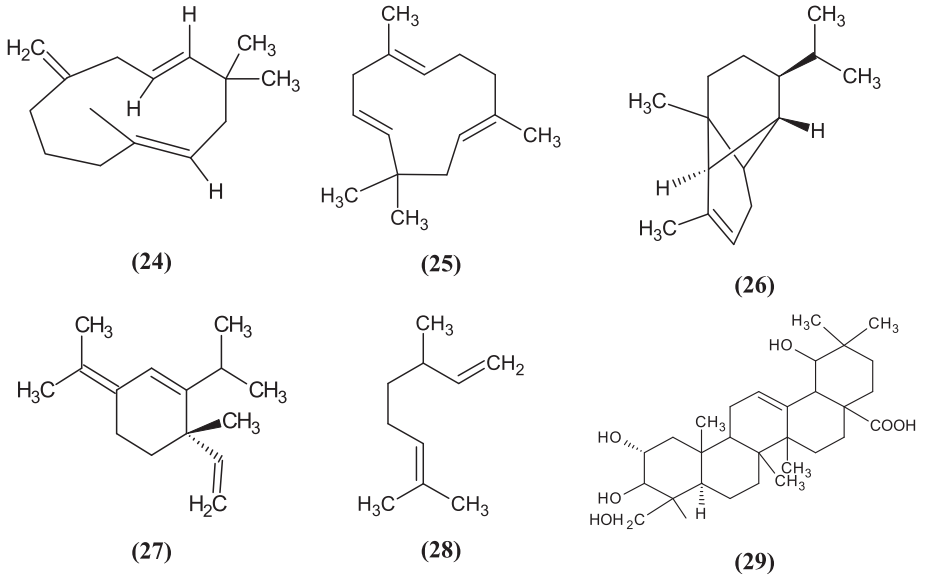

Fig. 4 Chemical structures of terpenes.

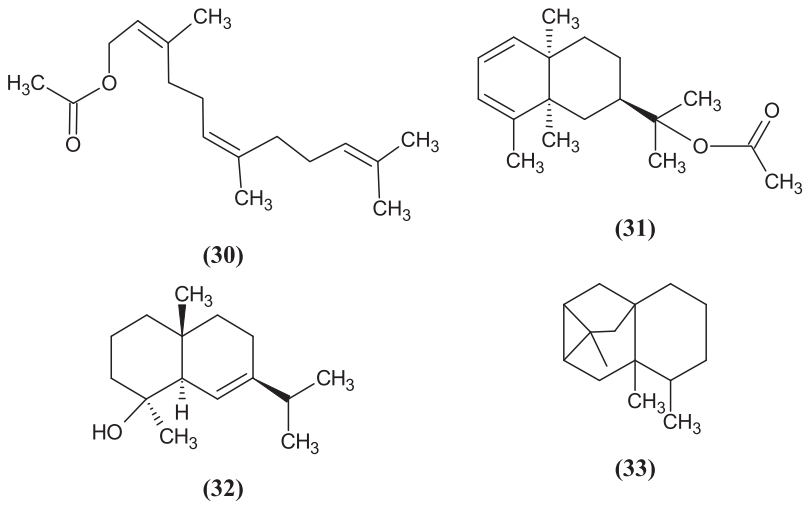

Fig. 5 Chemical structures of volatile compounds.

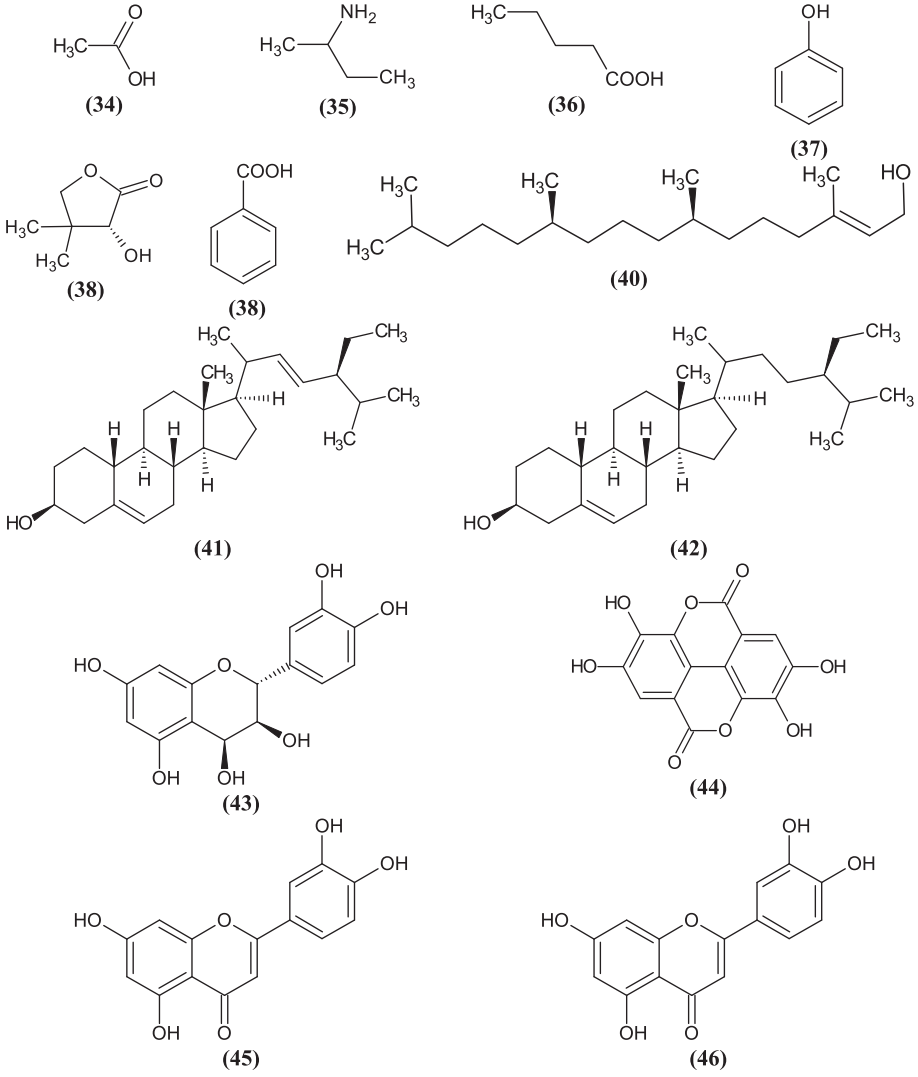

Fig. 6 Chemical structures of other compounds.

Table 2 Pharmacological effects of *Bixa orellana*.

Pharmacological effects	Details	Extracts/compounds	Potency of extracts/compounds zone of inhibition (mm)/ % Inhibition	MIC/dose level	*In vitro*/*In vivo*	References
Antibacterial and antifungal activity	• Broad spectrum antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* • Showed differential *in vitro* antimicrobial activity against *B. pumilus*	Ethanolic leaves and seeds extract	21.50, 20.00, 19.50, 17.00, 19.00, 22.50, 22.00 (leave extract) and 20.00, 17.00, 19.00, 14.50, 19.00, 18.00, 20.00 (seed extract), respectively.	–	*In vitro*	[32]
		Ethanolic leave extract	21.6	24 mg/mL	*In vitro*	[33]
		Ethanolic hypocotyls extract	15.8			
		Ethanolic root extract	15.20			
		Acetone extracts	10-14	–	*In vitro*	[14]
		DMSO extracts	> 14			
		Dichloromethane/ ishwarane	–	–	–	[37]
		Methanolic crude extract	17	–	*In vitro*	[3]
		Hexane extract		2.0 mg/mL		
		95% ethanol leaves extract	15–17	5 mg/mL	*In vitro*	[40]
Antioxidant and free radical scavenging activity	• Activity against reactive oxygen and nitrogen species (H_2O_2, HOCl, O_2, $^{\cdot}NO$, and $ONOO^-$ species)	Ethyl acetate extract, composed of hypolaetin and caffeoyl acid derivative	11.0, 1.0, 3.0 and 7.0, respectively	3.0 µg/mL	*In vitro*	[42]
	• antioxidant activity via 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and iron (III) oxide reducing power using ascorbic acid (vitamin C) as a reference standard	Seed extract	5.5–48.9% relative to ascorbic acid 2.9–41.5%	0.25 and 2.5 µg/mL	*In vitro*	[44]
		Bixin	33%	2.5 or 5.0 mg/kg	*In vitro*	[45]
	• Reduced total number of chromosome aberrations, inhibited the increase in lipid peroxidation, and renal glutathione depletion induced by cisplatin					
Anti-inflammatory activity	• Effect of aqueous extract of *Bixa orellana* on histamine-induced paw edema in rat models	Aqueous extract	–	150 mg/kg	*In vitro*	[46]
	• Significantly decrease carageenan, histamine, serotonin and bradykinin induced acute and chronic rat paw edema	Pretreatment of aqueous leaf extract	–	50 mg/kg and 150 mg/kg	*In vitro*	[46]
		After treatment of leaf extract (lyophilized)	–	50 mg/kg and 150 mg/kg	*In vitro*	[47]
	• Inhibits bradykinin-induced inflammation. and decreases nitric oxide production and vascular endothelial growth factor (VEGF)	Leaf extract	–	200 mg/kg and 400 mg/kg	*In vitro*	[48]
		Aqueous extract (2-butanamine, acetic acid, pentanoic acid, phenol, pantolactone and benzoic acid)	–	–	*In vitro*	[10]
	• Produced partial gastroprotective effects against 96% ethanol induced injury and reduced migration of pro-inflammatory cells					
	• Reduced paw volumes and almost normalized peritoneal vascular permeability, suspected to be aided by the suppression of other permeability-regulating substances (NO and VEGF)	Bixin	19% and 33.60%	50 µg/mL	*In vitro*	[50]
	• Inhibited COX-2 and COX-1 enzyme					

Table 2 (*continued*)

Pharmacological effects	Details	Extracts/compounds	Potency of extracts/compounds zone of inhibition (mm)/ % Inhibition	MIC/dose level	*In vitro*/*In vivo*	References
Anti carcinogenic activity	• The cell proliferation inhibitory effects against colon, CNS, stomach, and lung cancer cell lines	Bixin	–	33, 49, 45, and 39 μg/mL	–	[50]
		Methanol leaves extract	52%, 57% and 53%	500 mg/kg	*In vitro*	[52]
	• Decreased carbon tetrachloride hepatoprotection in rats with decrease in the elevations of liver alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol	Cis-bixin	–	10–50 μM	*In vitro*	[53]
	• Selectively killed freshly collected patient multiple myeloma cells and highly drug-resistant multiple myeloma cell lines					
Gastrointestinal motility	• Prophylactic and gastrointestinal motility	Dichloromethane extract of the air-dried leaves (Ishwarane)	(88.38 ± 13.59%)	25, 50, and 100 mg/kg	*In vitro*	[37]
	• Resulted in a more propulsive movement of the gastrointestinal tract	Ishwarane		50 mg/kg	*In vitro*	[37]
	• Delayed intestinal transit of charcoal meal in mice to a statistically significant level ($p < 0.01$)	Methanol leaves extract	79.55%	125, 250 and 500 mg/kg 500 mg/kg	*In vitro*	[54]
Neuropharmacological activity and Anticonvulsant activity	• Pentobarbitone-induced hypnosis test: Leaves extract was found to reduce the time for onset of sleep compared to the control with results statistically significant	Leaves extract		500 mg/kg	*In vitro*	[54]
		Leaves extract	58.45 min (control group) 76.70 and 90.82 min	250 and 500 mg/kg	–	[54]
	• Increases duration of sleep in test animals compared to the control					
	• Strychnine-induced anticonvulsant test: increased the average survival time	Leaves extract	7.33 and 10.68 min	250 and 500 mg/kg	–	[54]
Analgesic activity and Antidiarrheal activity	• Oral glucose tolerance test: lower blood glucose level when administered 45 min before glucose load	Methanol leaves extracts	43.60%	–	*In vitro*	[55]
		Methanol leaves extracts	–	22.36 μg/mL	*In vitro*	[54]
	• Inhibited caster-oil induced diarrhea in mice: Significant decrease in the total number of stools and dose-dependently the total number of feces and the total number of wet feces.					
Other pharmacological effects	• Diuretic effect with a significant increase in urine volume and levels of sodium, potassium and chloride	Methanol extract	–	500 mg/kg	*In vitro*	[58]
		Ethanol extracts	–	–	*In vitro*	[59,60]
	• Used to neutralize snake venom and prevents associated adverse effects	Whole plant extracts (Root and leaf extract)	6.0 and 17.40	–	*In vitro*	[61]
	• provide partial protection against the edema forming activity and lethality in mice against *Bothrops asper* and *B. atrox* venom and potent antigonorrheal activity					

species (MIC = 31.25 μg/mL) against 10 μg/disc Streptomycin (9 ± 0.3 mm and 20 ± 0.2 mm) used as control, and *Trichophyton mentagrophytes* and *Trichophyton rubrum* (18 ± 0.3 mm) against 10 μL/sample Amphotericin-B with 19 ± 0.3 mm and 32 ± 0.2 mm, respectively [29]. This was attributed to the presence of alkaloids in the leaf extract. Additionally, crude ethanol leaf extracts exhibited better antibacterial effects against *P. aeruginosa* (MIC 512 μg/mL) and *B. cereus* (MIC 4096 μg/mL) whereas MIC values of seed extract were 128 and 1024 μg/mL respectively, and results were compared to standard bacteriocin drug niacin [30]. Braga et al. studied activity of fruit extract against *Cryptococcus neoformans* with MIC value of 78.0 μg/mL compared to standard Amphotericin-B with MIC value of 0.078 μg/mL [31].

Ethanolic leaf and seed extracts of *B. orellana* showed broad spectrum antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*; the zone of inhibition (mm) was 21.50, 20.00, 19.50, 17.00, 19.00, 22.50, 22.00 (leaves extract) and 20.00, 17.00, 19.00, 14.50, 19.00, 18.00, 20.00 (seed extract) compared to 10 mg/ml gentamycin with MIC values of 34.00, 30.00, 24.00, 19.50, 33.00, 31.00, respectively. The results provide scientific support for the use of *B. orellana* in traditional medicine particularly as a gargle for sore throats and oral hygiene [32]. Ethanolic leaves extracts showed *in vitro* antimicrobial activity against *Bacillus pumilus* followed by the extracts from root and hypocotyls with zone of inhibitions of 21.60, 15.80 and 15.20 for 24 mg/mL concentration, respectively [33]. Analysis of dried leaves showed that a sesquiterpenes (Bixaghane) along with ellagic acid, 7-bisulfate luteolin, 8-bisulfate hypoluteolin, 7-glucoside luteolin, and bixorellin accounts for the potent antimicrobial activity of this plant [34]. The major antimicrobial compounds in the *B. orellana* seed extract were identified as carotenoids (9′-cis-norbixin and all-trans-norbixin) by ^{1}H NMR and screened by thin layer chromatography and bioautography followed by liquid chromatography/photodiode array/mass spectrometry (LC/PDA/MS) analysis [26]. In 2012, antibacterial activity of the ethanolic, methanolic, acetone and dimethyl sulphoxide extracts was evaluated against *E. coli*, *K. pneumonia*, *P. aeruginosa*, *B. subtilis*, *B. cereus* and *S. aureus* by disk diffusion assay. The antibacterial effects were more pronounced in acetone and DMSO extracts as compared to ethanol and methanol extracts with zone of inhibition 10–14 mm and >14 mm, respectively against 25 μg tetracycline used as standard [12]. The crude extract of *B. orellana* hairy roots was assessed for anti-plasmodium activity against malaria strains 3D7 and K1 and displayed antimalarial properties in the 15–20 μM range with no cytotoxicity at the measured concentrations in the mammalian cell lines utilized for this experiment (EC50 > 26 μM) [35].

Previous investigations demonstrated the antifungal activity of the essential oils obtained from leaf extract of this plant against a wide variety of fungal strains [36]. The antifungal activity of sesquiterpene ishwarane, isolated by dichloromethane extraction had an activity index of 0.3 against *C. albicans* and *T. mentagrophytes* [37]. Phytochemical investigation revealed that alkaloids, tannins, triterpenoids and anthraquinones are responsible for the antifungal activity. Hexane extract showed antifungal effects against *Trychophyton mentagrophytes* and *Trychophyton rubrum* with MIC value of >8.0 mg/mL [38], and the results are comparable to earlier studies of antifungal activity against *Cryptococcus neoformans* and *Microsporum gypseum* with MIC values of 8 and 2 mg/mL, respectively [39]. In addition to above, 95% ethanolic leaves extract (5 mg/mL) of *B. orellana* was examined for *in vitro* antifungal and antibacterial activities using agar diffusion and tube dilution methods with zones of inhibition of 15–17 mm for all the standard strains of Gram-positive bacteria including *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus faecalis* while showing slight action against *Escherichia coli*, *Serratia marcescens*, *Candida utilis*, and *Aspergillus niger* while chloramphenicol and phenol positive controls show 12–18 mm and 10–28 mm, respectively [40].

Antioxidant and free radical scavenging activities

Conrad et al. investigated free radical scavenging potentials of ethanolic leaf extract of *B. orellana* [41]. Results indicate that phytochemical present in *B. orellana* leaves is effective protecting agents against carbon tetrachloride induced intoxication of blood and liver to albino rats. *In vitro* scavenging activity of various organic and aqueous seed extracts was evaluated against reactive oxygen and nitrogen species and results were compared to bixin standards. The results showed that ethyl acetate extract, mainly composed of hypolaetin and caffeoyl acid derivative (PC) had significant antioxidant effects with IC_{50} value of 11.0, 1.0, 3.0, 7.0 and 3.0 μg/mL as compared to bixin standards with IC_{50} values of 3.0, 0.3, 1.0, 3.0 and 1.0 μg/mL against H_2O_2, HOCl, O_2, NO, and $ONOO^-$ [42]. With increase in the polarity of the solvent, free radical scavenging activity of *B. orellana* extract increases which is in accordance with the presence of higher phenolic content in more polar solvents [43].

In *vitro* antioxidant activity of seed extract of *B. orellana* was tested via 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and iron(III) oxide reducing power using ascorbic acid (vitamin C) as a reference standard. Results indicated that the percentage reduction ranged from 5.5% to 48.9% relative to ascorbic acid (2.9–41.5%) between concentrations range of 0.25 and 2.5 μg/mL. Similarly, iron(III) oxide reducing power shows good linear concentration-dependent relation (R^2 = 0.9986) comparable with ascorbic acid (R^2 = 0.9934). According to the authors activity may be due to the presence of tannins and flavonoids found in the preliminary analysis [44]. Pretreatment with bixin (2.5 or 5.0 mg/kg BW; 48, 24 h and 10 min) reduced the total number of chromosome aberrations by about 33%, inhibited the increase in lipid peroxidation, and inhibited renal glutathione depletion induced by cisplatin (5 mg/kg BW) [45].

Anti-inflammatory activity

The results of the inhibitory effect of aqueous extract (150 mg/kg) of *B. orellana* on histamine-induced paw edema in rat models showed reduced histamine-induced paw edema in a dose dependent manner [10]. Additionally, pretreatment of 50 mg/kg and 150 mg/kg of aqueous leaf extract could significantly decrease carageenan, histamine, serotonin and bradykinin induced acute and chronic rat paw edema [46]. After treatment with lyophilized leaf extract at dose levels of 50 mg/kg and 150 mg/kg inhibits bradykinin-induced inflammation. Also a decrease in nitric oxide production and vascular endothelial

growth factor (VEGF) was observed indicating that the anti-inflammatory effect may be related to the reduction in reactive oxygen species [47]. The antiulcer effects of hydroalcoholic leaf extract were studied on rat liver damage induced by 96% ethanol. The leaf extract at doses of 200 mg/kg and 400 mg/kg produced partial gastroprotection [48].

Results from the inhibitory effect of aqueous extract of *B. orellana* leaves on paw inflammation induced by histamine in mice showed significant reduction in paw volumes and almost normalized peritoneal vascular permeability, suspected to be aided by the suppression of other permeability-regulating substances (NO and VEGF) [10]. GC/MS analysis showed the presence of six major components 2-butanamine, acetic acid, pentanoic acid, phenol, pantolactone and benzoic acid, although results from the studies by Ruiz and comes showed that low concentration of acetic acid (0.3 mg/mL) has been shown to inhibit histamine release from guinea pig lung mast cells when stimulated by both antigen (ovalbumin) and ionophore A23187 [49]. Additionally, bixin (50 μg/mL) from *B. orellana* was screened for COX-1 and COX-2 enzyme inhibitory activities showing 19% and 33.60% inhibition, respectively compared with ibuprofen (2.52 μg/mL), aspirin (180 μg/mL), Vioxx (1.67 μg/mL), and Celebrex (1.67 μg/mL), used as positive controls, giving 51, 78, 63, 0.7 and 40%, 99%, 32% and 82% inhibition, respectively [50].

Anticarcinogenic activity and cytotoxicity-

Extracts and compounds from *B. orellana* also possessed anticancer/antitumor effects. The main effective compound was thought to be bixin [50]. The cell proliferation inhibitory effects of bixin varied among tumor cell lines giving respective IC_{50} values of 33, 49, 45, and 39 μg/mL against colon, CNS, stomach, and lung cancer cell lines. Clastogenic and anticlastogenic activity of bixin from seeds of *B. orellana* was evaluated in order to assess the chromosomal damage induced by the clastogen cisplatin [51]. Methanol leaf extract (500 mg/kg, crude herb medicinal equivalent, 3 times/day) decreased carbon tetrachloride hepatic damage in Swiss albino rats. Decrease in the elevations of liver alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol by 52%, 57% and 53%, respectively has been monitored and supported by histopathological examination of liver tissues [52].

Cis-bixin from dried annatto seeds induces cytotoxicity in a wide variety of tumor cell lines in an *ex vivo* myeloma model system with IC_{50} values of 10–50 μM, 24 h exposures, and selectively killed freshly collected patient multiple myeloma cells and highly drug-resistant multiple myeloma cell lines [53]. The cis-bixin induced cytotoxicity was thought to be induced by ROS in dose- and time-dependent manner as demonstrated by the inhibition of thioredoxin and thioredoxin reductase and fluorescence-activated cell sorting (FACS) assays using the cell-permeable dyes 5-(and-6) chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester or dihydroethidium.

Gastrointestinal motility

A bioactive sesquiterpene (ishwarane) was isolated from dichloromethane extract of the air-dried leaves of *B. orellana* by silica gel chromatography and identified by ^{1}H and ^{13}C NMR. At dose levels of 25, 50, and 100 mg/kg ishwarane was tested for gastrointestinal mobility. Results of the prophylactic assay showed the antitoxic property at 100 mg/kg. At a dose of 50 mg/kg ishwarane resulted in a more propulsive movement of the gastrointestinal tract (88.38 ± 13.59%) as compared to negative control (78.47 ± 10.61%) [36]. A methanol leaf extract (125, 250 and 500 mg/kg, 30 min) was found to delay the intestinal transit of charcoal meal in mice to a statistically significant level ($P < 0.01$) with 79.55% inhibition at a dose of 500 mg/kg [54].

Analgesic activity and hypoglycemic activity

Analgesia of isolated ishwarane from *B. orellana* leaves showed 47.93 ± 15.16% inhibition relative to diclofenac sodium as positive control 42.53 ± 37.75% in tail flick model and an inhibition of 60 ± 41.22% relative to positive control 56.84 ± 32.60% in acetic acid writhing assays [36]. Methanol leaf extract showed hypoglycemic effects (30.2%, $P < 0.040$) in Swiss Webster mice after 45 min of loading glucose using a modified oral glucose tolerance test [55].

Antidiarrheal activity

Results from the study by Shilpi et al. showed that the methanol leaf extract (125, 250 and 500 mg/kg BW) inhibited castor oil induced diarrhea in mice. Antidiarrheal activities were supported by a statistically significant decrease in the total number of stools (including wet stools) and dose-dependently the total number of faces and the total number of wet faces with IC_{50} value of 22.36 μg/ml [54].

Other pharmacological effects

Methanol extract showed potent activity against *L. amazonensis* isolated from a patient with diffuse cutaneous leishmaniasis with IC_{50} = 22 μg/mL [56]. Additionally further research findings on *in vitro* and *in vivo* effects of the essential oil (Ishwarane and geranylgeranoil) of *B. orellana* seeds showed potential activity against intracellular amastigote form with IC_{50} value of 8.5 μg/mL [57]. The effect of methanol extract of *B. orellana* leaves on diuretics was demonstrated in Wister rat models, and results showed that the extract at a dose level of 500 mg/kg possessed diuretic effect with a significant increase in urine volume (2.4 ± 0.02 ml) and levels of sodium (82 ± 3.07 mEq/L), potassium (12.3 ± 0.47 mEq/L) and chloride (71 ± 2.52 mEq/L) as compared to control group 0.7 ± 0.04 mL, 62 ± 2.01, 11.4 ± 1.90 and 56 ± 1.90, respectively [58]. Additionally, various extracts of this plant have been used to neutralize snake venom and prevents associated adverse effects [59] and prove its use in folk medicine. Also, ethanol extracts (LD_{50} = 44 μg) offer partial protection against the edema forming activity and lethality in mice against *Bothrops atrox* venom [60]. Root and leaf extract of this plant have been found to have potent antigonorrheal activity with zones of inhibition 6.0 mm and 17.40 mm respectively [61].

The foliage of Bixa is used to treat skin problems and hepatitis, and also used as aphrodisiac, antidysenteric, and antipyretic [62]. The binding of naringenin-7-O-glucoside isolated from the fruit shell of *B. orellana* with calf thymus DNA (ctDNA) and the influence of cyclomaltoheptaose

(b-cyclodextrin, b-CD) on the binding were studied by absorption and fluorescence spectroscopic techniques [63].

Industrial uses of annatto dye

Before describing the potential industrial applications of annatto dye, we find useful to briefly explain how natural colorants including annatto have been reintroduced into textile coloration and other application fields. Since the introduction of synthetic dyes in 1856, use of natural colorants from plants had almost vanished [64–66]. However, during the past decade synthetic dyes especially those producing any of the banned aromatic amines upon their degradation were shown to possess some drawbacks mainly carcinogenicity and environmental pollution [67,68]. Therefore, the desire for green labeled products has led to a renaissance in natural colorants for use in textiles, food, cosmetics, dye synthesizer solar cells and other application fields [69,70,7]. Currently, a number of colorants from plant sources such as orange peel [71], pomegranate peel [72], almond shell [73], gallnut [74], *Hibiscus mutabilis* [75], *Terminalia arjuna* [76], *Terminalia catappa, Saraca asoca* [77], *Tectona grandis* [78], tea, turmeric [79], betanin [80], madder [81], weld [82], henna [83,84], and chestnut shell [85] have been investigated as potential candidates for sustainable coloration and functional finishing of different kinds of textile materials. Annatto is one such natural yellow–orange dye obtained from renewable resource such as *Bixa orellana* plant, causes less toxicity and generally exhibits better biodegradability and compatibility with the environment.

Currently, the European Union has authorized a vast number of natural colorants as food additives including annatto which is assigned E-number of E160b for use in a wide range of food commodities such as dairy products, flour confectionery, fish, soft drinks, meat products, snack foods, and dry mixes [64,86]. Despite its numerous food applications it has shown huge potential in textile and leather sectors. Bixin (Cis-form) is the principle coloring compound present in annatto seeds. It is oil soluble diapocarotenoid with two carboxylic acid groups in its molecular structure one of which is esterified and accounts for more than 80% of total annatto pigments [87,88]. Another carotenoid type water soluble coloring compound is nor-bixin which is derived from bixin by hydrolysis of the ester group [89,90].

Numerous approaches are used to extract the pigment from dried annatto seeds among which direct extraction using oil, aqueous alkali, or indirect extraction with solvents has been commercially accepted methods of bixin and nor-bixin extraction [91]. In view of its emerging importance, new green techniques have been employed for the extraction of annatto dye from *Bixa orellana* seeds. Sinha and colleagues reported microwave-assisted extraction of yellow–red from seeds by investigating the effects of pH, extraction time and amount of annatto seeds using response surface methodology (RSM) and artificial neural network (ANN) based predictive methods [92]. Lately, Yolmeh et al. studied ultrasound method for extraction of annatto dye. They used a statistical approach based on response surface methodology to optimize the extraction conditions such as concentration of solvent, extraction time, duty cycle, and solvent-to-material and found that ultrasound technology offers much better results than conventional techniques [93].

Several researches have been undertaken on the dyeing of textiles with annatto. Reports are available in the literature on dyeing properties of bixin on synthetic textile materials such as nylon and polyester [94]. Likewise, annatto pigments have demonstrated better results after their application on natural fibers including wool, silk and cotton [95,96]. Recently, annatto dyed wool post-treated with ammonia resulted in a variety of beautiful shades with variation in hue and tone [69]. In 2015, Selvi et al. investigated the dyeing potential of four plants found in Mexico including *B. orellana* [97]. Among all the plants studied *B. orellana* was found to be less toxic when used to dye cotton cloth and manta without a chemical mordant.

B. orellana has been successfully introduced in leather dyeing and finishing. For the first time Selvi and coworker studied the feasibility of using natural dye extract from *B. orellana* seeds for dyeing and finishing of leather. The leathers dyed using the annatto extract showed better coloring and satisfactory fastness properties and hence offer a viable option for commercial exploitations as a replacement for synthetic dyes and pigments [98]. In another research work, Siva and coworkers reported a study to discover the potential of pigment from *B. orellana* as an alternative tracking gel to bromophenol blue for electrophoresis [99]. Bromophenol blue is a synthetic dye and could produce allergic and toxic reactions. They observed encouraging results for annatto dye with the developed procedure being easy, practical and reliable.

Chemical structure is an important component for efficient functioning of any dye sensitized solar cell. Over the past fifteen years there has been interesting exploration of natural colorants from flowers, seeds, fruits and leaves as possible ecofriendly and low cost sensitizers [100,101]. Recent research on annatto has resulted in utilization of its pigments as sensitizer for dye sensitized solar cells. Haryanto and coworkers studied the manufacture of dye sensitized solar cell (DSSC) using annatto seeds and found quite satisfactory efficiency of fabricated solar cells [102]. Likewise, Gomez–Ortiz studied the use of bixin and nor-bixin in dye-sensitized solar cells (DSCs) and obtained efficiencies of up to 0.53% by using bixin-sensitized TiO_2 solar cells [70].

Conclusion and future perspectives

This review summarizes recent research into the phytochemistry and pharmacology of *Bixa orellana* plant. Many of the ethnopharmacological uses and biological activities have been validated by *in vitro* studies and *in vivo* models. Although studies conducted on annatto have confirmed its wide-range of biological activities, more rigorous research is further needed to explore individual chemical constituents and their action mechanisms in exhibiting certain biological and pharmacological activities in order to introduce this plant in pharmaceutical and other industries. Most of the studies have proved that annatto extracts have potent biological activities compared to standard drugs tested which further encourage scientists to deepen the investigations in order to develop safe and effective drugs from this plant in the near future.

Furthermore, in the last part this review has documented the presence of carotenoids mainly bixin and nor-bixin in annatto seeds which have yellow coloring properties. The utilization of the seed extract as natural colorant is of commercial

value in USA and is permitted by FDA for use in food and drinks. It exhibits a high potential for use in a wide range of food commodities such as dairy products, flour confectionery, fish, soft drinks, meat products, snack foods, and dry mixes. Efforts have been made in the recent years to introduce this colorant in other non-food sectors such as dyeing industry and leather finishing and as a novel sensitizer for dye sensitized solar cells. For commercial utilization in non-food applications, more researches on annatto dye chemistry and extraction methods need to be further and preciously explored in order to exploit full potential of this natural dye plant.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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