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# PHOTOCHEMISTRY, TRADITIONAL AND PHARMACOLOGICAL USES OF BIXA ORENALLA: A REVIEW

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## ABSTRACT
*Bixa orellana* has been used by indigenous communities in Brazil and other tropical countries for several biological applications, which indicates its potential use as an active ingredient in pharmaceutical products. Bixa having great agroindustrial value because its seeds have a high carotenoid content, mainly bixin. BIxa is commercially known as annatto and It is currently used as a natural pigment in the food, in pharmaceutical, and cosmetic industries. 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, hypoglucemic, antibiotic and antiinflammatory 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.

**KEYWORDS:** anti-cancer, hypoglucemic, antibiotic and anti-inflammatory.

## INTRODUCTION
Bixaorellana is a plant native to Brazil but these also found in other regions of South and Central America. These plants also grow in the tropical Countries such as Peru, Mexico, Ecuador, Malaysia, Indonesia, India, Kenya, and East Africa. Native cultures in underdeveloped nations use Bixa orellana L., sometimes known as achiote or annatto (family: Bixaceae). (Gamble, 1957) as folk medicine for the treatment of common illnesses in the form of decoctions, teas, and juices. Bixa Orenella is belonging to Bixaaceae family which is one of the smallest families, consisting only of one genus Bixa. There are only five species grouped under a single genus, and the most common species is Bixa orellana, an evergreen shrub grown not only because of its beautiful red flowers and ornamental red spiny fruits, but also for its economic value. The Annatto seed extract contains many color principles among all bixin, oil soluble and norbixin, water soluble principles are responsible for its dye characteristics.[4] Bixin responsible for imparting reddishness and norbixin for yellow. Annatto color imparts yellow to red with varied hue index as it possesses high tinctorial value, hence have significance in the food industry as a natural food grade colour, and stands second in rank among economically important natural food colourants. The main actions of annatto color are it kills bacteria, parasites, germs, increases urination, stimulates digestion, lowers blood pressure,mildly laxative, and protects liver.[5] The other actions of annatto color includes it reduces inflammation, cough,Cleanses blood, soothes membrane, reduces fever, blood sugar, heals wounds. Bixahas also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardio protective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions.[6] The use of natural remedy for burn wounds is common in tropical countries such as the Philippines. These applied traditionally to burn wounds for immediate and continued Relief.

## Botanical
The annatto tree belongs to the family Bixaceae and thegenus Bixa. Despite the existence of several species, the mostcommon in our country is Bixa orellana L., named after Francisco Orellana, who was the first European to navigatethe Amazon. B. orellana is a small tree or shrub measuring from 3 to 5 meters in height, sometimes reaching a height of 10 meters. The trunk is short, measuring 20–30 cm in diameter, with dark gray bark with lenticels in vertical rows. The leaves are alternate, 10 to 20 cm long and 5 to 10 cm wide, sharp, green on both sides, and with extended petioles. Seeds measure 0.3–0.5cm in length and 0.2-0.3 cm in diameter and their shape varies from pyramidal to almost conical.

The seeds are considered the plant part of commercial importance, since the pericarp (layer that surrounds the seeds) contains the pigments that have wide industrial application.

About 80%of this pigment is the carotenoid known as bixin, which has the dye property and can be extracted with vegetable oils or chemical bases. Depending on the cultivar and climatic conditions of the region, the bixin content canvary from1 to 6%in the seed aril. The remainder is composed of other dyes and inert substances of minor importance.

**Photochemistry**

**CHARACTERISTICS AND PROPERTIES OF BIXIN**

Bixin is a lineal apocarotenoid of 25 carbon particles with 9 twofold bonds and a sub-atomic load of 394.5 g/mole (Francis, 1987; Britton et al., 2004); its molecular empiric formula is C25H30O4, and its scientific name is methyl hydrogen 9 0 -cis-6,60 -diapocaroteno-6,60 -dioate ester (Preston and Rickard, 1980; Mercadante et al., 1996). Seed Extract of Bixa contains variety of apocarotenoids, including both linear (i.e., methyl (9 Z)-apo-80 -lycopenoate) and cyclic molecules (all-E)-80 -apo-β-caroten-80 -oate). These apocarotenoids are terpenoid compounds derived from the oxidative cleavage of carotenoids. Lycopene is described as bixin precursor (Figure 1B), although it may also have other non-studied biosynthetic pathways, since bixin is produced despite the inhibition of carotenogenesis using the highest concentration of norfluorazon, a phytoene desaturase inhibitor (Rivera-Madrid et al., 2013). Bixin has two different stereochemical configurations: cis bixin and trans-bixin. The former cis is soluble in most polar organic solvents to which it imparts an orange color and is largely insoluble in vegetable oil (Mckeown and Mark, 1962; Francis, 1987; Scotter, 1995; Scotter et al., 2002). It may be readily converted to the all-trans-isomers due to the instability of the isolated form in solution. Trans-bixin is a more stable isomer, it exhibits a red color in solution and is soluble in vegetable oil (Francis, 1987; Scotter, 2011).

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.

- **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-(90 Z)-6,60 -diapocarotene-6,60 -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. isolated b-carotene, cryptoxanthin, lutein zeaxanthin ,and methyl bixin in addition to bixin and nor-bixin from seeds by thin layer chromatography. Chemical investigation of methanol seed extract has resulted in the identification of the apocarotenoids methyl bixin.

- **Terpenoids and terpenes**

Terpenoids mainly C20-terpene alcohol all-geranylgeraniol as a major chemical component in Bixa orellena were isolated by Jondiko and Pattenden. Other terpenes that were isolated and characterized for the first time include farnesylacetone[19], geranylgeranyl octadecanoate[20], geranylgeranyl formate[21], d-tocotrienol[22] and b-tocotrienol.[23] Frega et al. reported the presence of tocotrienols mainly dtocotrienol from lipid fraction of annatto seeds using thinlayer chromatography. Sesquiterpenes are also a major group of volatile compounds found in annatto extracts In one of the recent studies on annatto b-humulene[5] 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 a-copaene[26], and a-elemene.[27] The chemical structures for all the isolated terpenes and terpenoids 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 S.ul-Islam et al. 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-penten2-one, 4-hydroxy-4-methyl-2-pentanone, 6-methyl-5-hepten2-one, acetic acid, ethyl butyrate, 1,2-propanediol-2-acetate, 3-methylpyridine, p-xylene and toluene, d-elemene, a-pinene, limonene, b-myrcene, eucalyptol, b-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%).

- **Other miscellaneous compounds**

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[6], pantolactone[38] and benzoic.[39] 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.

**Table No. 01. Chemical Constituents in Bixaorellana.**

| Sr.No. | Classification | Components | Plant part |
|---|---|---|---|
| 1 | | Methylhydrogen-(9$^0$Z)-6,6$^0$-diapocarotene-6,6$^0$-dioate(Bixin) | Seedcoat |
| 2 | | Dimethyl-(9Z,9$^0$Z)-6,6$^0$-diapocarotene-6,6$^0$-dioate | Seedcoat |
| 3 | | Methyl(9$^0$Z)-apo-6$^0$-lycopenoate | Seedcoat |
| 4 | | Methyl-(7Z,9Z,9$^0$Z)-apo-6$^0$-lycopenoate | Seedcoat |
| 5 | | Methyl-(9Z)-apo-8$^0$-lycopenoate | Seedcoat |
| 6 | | Methyl-(all-E)-apo-8$^0$-lycopenoate | Seedcoat |
| 7 | | Methyl-(all-E)-apo-6$^0$-lycopenoate | Seedcoat |
| 8 | | Methyl(9*Z*)-10$^0$-oxo-6,10$^0$-diapocaroten-6-oate | Seeds |
| 9 | **Carotenoids** | Methyl(9*Z*)-6$^0$-oxo-6,5$^0$-diapocaroten-6-oate | Seeds |
| 10 | | Methyl(9Z)-6$^0$-oxo-6,6$^0$-dioapocarotene-6-oate | Seeds |
| 11 | | Methyl-(4Z)-4,8-dimethyl-12-oxododecyl-2,4,6,8,10-pentaenoate | Seeds |
| 12 | | 6-Geranylgeranyl-8$^0$-methyl-6,8$^0$-diapocaroten-6,8$^0$-dioate | Seeds |
| 13 | | 6-Geranylgeranyl-6$^0$-methyl(9$^0$*Z*)-6,6$^0$-diapocaroten-6,6$^0$-dioate | Seeds |
| 14 | | 6-Geranylgeranyl-6$^0$-methyl-6,6$^0$-diapocaroten-6, 6$^0$-dioate | Seeds |
| 15 | | Trans-bixin | Seeds |
| 16 | | Farnesylacetone | Seeds |
| 17 | | Geranylgeranyloctadecanoate | Seeds |
| 18 | **Terpenoids** | Geranylgeranylformate | Seeds |
| 19 | | d-Tocotrienol | Seeds |
| 20 | | b-Tocotrienol | Seeds |
| | | | |
| 21 | | b-Humulene | Roots |
| 22 | | a-Carpophyllene | Leaves and roots |
| 23 | **Terpenes** | a-Copaene | Leaves and roots |
| 24 | | a-Elemene | Leaves |
| 25 | | Cis-ocimene | Leaves |
| 26 | | Tomentosicacid | Roots |
| | | | |
| 27 | | (Z,E)-farnesylacetate(11.6%) | Seed oil |
| 28 | **Volatilecompounds** | Occidentalolacetate(9.7%) | Seed oil |
| 29 | | Spathulenol(9.6%) | Roots, seedoil |
| 30 | | Ishwarane(9.1%) | Seed oil |
| | **Othercompounds** | | |
| 31 | | Aceticacid | Roots |

| | | | |
|---|---|---|---|
| 32 | | 2-Butanamine | Roots |
| 33 | | Pentanoic acid | Roots |
| 34 | | Phenol | Roots |
| 35 | | Panto lactone | Roots |
| 36 | | Benzoic acid | Roots |
| 37 | | Phytol | Leaves |
| 38 | | Stigmasterol | Leaves |
| 39 | | Sitosterol | Leaves |
| 40 | | Leucocyanidin | Leaves |
| 41 | | Ellagicacid | Leaves |
| 42 | | Luteolin | Leaves |
| 43 | | Apigenin | Leaves |
| | | | |

**BIXIN BIOSYNTHESIS IN Bixa orellana**

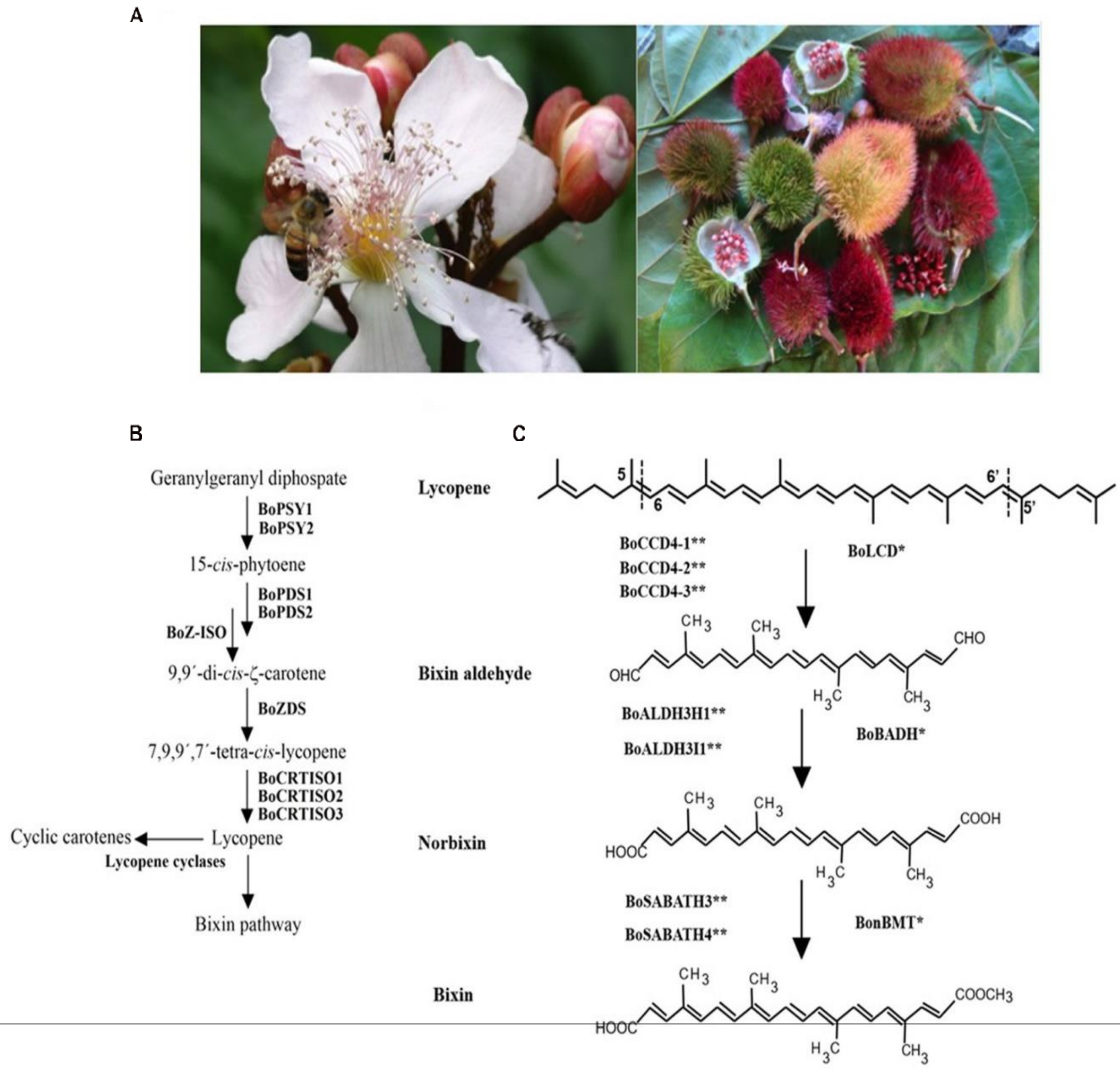
Plant carotenoids is the most important because of they have a crucial role in photosynthesis which helping to collect light and conferring protection against its excess. Carotenoids are also important precursors of bioactive compounds, such as apocarotenoids which are important in several physiological 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 50–60 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. 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 similar to 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 bacterias 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, CárdenasConejo 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.




**Bixin biosynthesis pathway**
**(A)*Bixaorellana*L.buds,flower(left),fruitandseeds(right).**
**(B)CarotenoidbiosynthesisPathway.**
**C)Bixinbiosynthesispathway.**

**Use in Traditional Medicine**

- **Medicine**

The plant and its parts are widely used in the traditional medical system in various parts of world (Shahid-ul-Islam et al., 2016, Venugopalan et al., 2011). The plant has been used since precolonial times as a culinary colorant and spice, and for healing purposes (Rivera-Madrid et al., 2016) and treating infections of microbial origin (Ganju and Ganju, 2014, Rojas et al., 2006). In Central and South America plants are used to treat internal inflammation and in Malaysia for gastric ulcers and stomach discomfort (Yong et al., 2013). In India the bark are used to treat fever, gonorrhoea phlegm, blood diseases, headache (Kumaran, 2014), and there are evidences that plant do not show any type of urogenital infections while treating gonorrhea (Stohs, 2014).

The seeds are slightly purgative and possess nutritive value and in West Africa, New Guinea, Guyana they are effective against dysentery, fever and kidney disorders and used in poisoning by cassava (Jansen, 2005). The Amazon tribes of Brazil generally use seed extracts to paint their body which also claims to repel insects (Giorgi, 2013) as evidenced through studies in Wajapi tribal women of Brazil (Mata et al., 2012). In Gabon leaf decoction is used against vomiting, in DR Congo used as a gargle for sore throat and tonsillitis and treating itches, in Seychelles and Mauritius as a bath against muscular pain and headache (Jansen, 2005), in Trinidad and Tobago to treat diabetes, jaundice and hypertension (Lans, 2006). In Mauritius the leaves are used against headache and in Ethiopia applied as a wound dressing. In Paraguay and Mexico mixture of seeds and sap are used against mouth disorders (Jansen, 2005).

- **Food industry**

The natural biocolorants yellow or orange colors bixin obtained from the seeds are safer for food use and exempted from certification category of FDA and EU (Aberoumand, 2011). These natural colorants are used in food industry in many countries (Ganju and Ganju, 2014, Dequigiovanni et al., 2018) mainly in dairy, confectionary and bakery products (Shahid-ul-Islam et al., 2016, Venugopalan et al., 2011, Srineeraja, 2017). The dye is non-toxic with traces of vitamin A content and soluble in lipids hence used to colour margarine, ice-cream, candy, cheese, butter, bakery products and oils. In Latin America, annatto is used to give red colour to fish, meat, and rice dishes and vegetable dyes as a turmeric supplement (Jansen, 2005, Galindo-Cuspinera et al., 2002).

- **Cosmetic and textile industries**

Annatto is a natural dye yielding plant and has applications in textile and cosmetic industries, leather, solar cells and other industries (Shahid-ul-Islam et al., 2016, Dequigiovanni et al., 2018). Artificial neural network (ANN) shows high degree of potentiality in optimizing the dye extraction from seeds of the plant (Sinha et al., 2013). Annatto dye has significant uses in cosmetic and leather industry (Venugopalan et al., 2011, Gupta, 2016). It is used in the cosmetic industry in the production of lipstick, hair oil, nail gloss, soap and in household products like furniture polish, floor wax, brass lacquer, shoe polish, and wood stain (Jansen, 2005). In textile industry annatto has been used for dyeing wool, cotton and silk, giving an orange-red colour and when mixed in dye-bath with wood-ash or sodium carbonate and treated with tartaric or citric acid, turns yellow (Jansen, 2005). According to some reports the woolen yarn dyed with annatto seeds shows decrease in lightness value on treatment with ammonia (Shahid-ul-Islam et al., 2014). The dye fades in sun light but resistant to soap, acids and alkalis. The dye is also used to colour wood, rattan and bamboo.

- **Other uses**

The press cake of the seed and fruit is used as fodder. The wood is used as good firewood and bark fibres are used for cordage (Jansen, 2005). In West Africa, the Baoulé community of Côte d'Ivoire uses crushed seeds paste mixed with lemon juice and water for painting toys, wooden masks and door posts (Jansen, 2005). There are also the reports of the use of natural dye in leather (Selvi et al., 2013), solar cells and other industries (Shahid-ul-Islam et al., 2016, Dequigiovanni et al., 2018). The fruit pericarp, a byproduct of colour extraction industries has potentiality as a biofuel as it shows better fuel value index than other plant biomass fuel sources (Parimalan et al., 2007.

- Hepatoprotection Methanol extract of B. orellanaseeds illustrated hepatoprotective activity against liver damage induced by carbon tetrachloride ($CCl4$)[6] B. orellana, showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells.
- ANTIMALARIAL ACTIVITY has been determined against Plasmodium gallinaceum, Plasmodium lophurae, falciparum and Plasmodium berghei.[12] B. orellanaextracts possess antiprotozoal, anthelmintic and plateletantiaggregant activity.[10]
- Diabetes Mellitus Bixaorellanahas been used for the treatment of diabetes mellitus. B. orellanalowered blood glucose by stimulating peripheral utilization of glucose,[41]. B. orellana had antihistamine activities anti-inflammatory activity anticonvulsant activity antidiabetic activity.[42-44]
- Antimicrobial Activity Bixaorellanashowed a broad spectrum of antimicrobial activity.[45][46] B. orellanain traditional medicine used as a gargle for sore throats and oral hygiene.Seedsurucum is used as a condiment as well as laxative, cardiotonic, hypotensive, expectorant, and antibiotic.[47-49] It has anti-inflammatory activity for bruises and wounds and has been used for the treatment of bronchitis and for wound healing purposes. Oil is also obtained from this plant. The infusion of the leaves has been shown to be effective against bronchitis, sore throat, and eye inflammation.[50] Bixaorellanaleaves exhibits antifungal activity.[51]
- Antimutagenic Activity It is potential against chromosomal damage induced by radiation[59] and clastogenic effects of antitumor agents[9] suggesting it as a promising agent against radiations. Bixaorellanaextract are good radioprotectors of bone marrow at non-toxic dose suggests that it may be promising agents for human radiation

  The protective effect of Bixaorellanaagainst DNA damage induced by UV radiation, hydrogen peroxide and superoxide anions promoted us to go assessing it‟sradioprotective potential at chromosomal level, and it also displayed antimutagenic properties, It thus has antigenotoxic properties and chemo-preventive effects.
- Antioxidant Annatto has been reported to contain tocotrienols (T3), a less prominent isomer of vitamin

E which has been reported to possess in vitro and in vivo anti-cancer activity in mutagenic rodents and this was recently confirmed via oxidative effect, senescent-like growth inhibition and immune modulation effect as well as in tumoral mammary glands of transgenic mice expression of HER-2/neu. Anti-apoptotic effect of δT3 and γ-T3 components of annatto have been established in vitro in human and mice tumor cell lines. Among the natural carotenoids, bixin is one of the more effective biological singlet molecular-oxygen quenchers and may contribute to the protection of cells and tissues against deleterious effects of free radicals.[54] Bixin is also an effective inhibitor of lipid peroxidation[55], inhibited TBARS production in peripheral macrophages, and this could be the mechanism by which carotenoids in vivo protect cells and tissues from damage induced by oxygen metabolites.[56] It is an antioxidant inhibitor of lipoxygenase activity.[57] Methylbixin has shown enhancement activity of gap junctional communication which is important in cancer prevention.

**Traditional uses of annatto in American countries**

| Country/use | Plant part |
|---|---|
| Argentina | |
| Antipyretic/cardiotonic/antidiarrheal | Seeds |
| Antidiarrheal/dyes/condiment | Seeds |
| Brazil<br>Bodypaint | Seeds |
| Insectrepellent | Seeds |
| Condiment/foodcoloring | Seeds |
| Antipyretic | Seeds |
| Antipyretic/laxatives/burns | Seeds |
| Malaria | Seeds |
| Colombia | |
| Snakebite | Leaves |
| Aphrodisiac | Seeds |
| Aphrodisiac | Seeds |
| Gonorrhea/dysentery | Leaves |
| Hepatitis | Leaves |
| Dysentery | Leaves |
| Blooddiseases | Leaves |
| Diabetes | Roots |
| Honduras<br>Aromatic/foodcoloring | |
| Pain/digestive/dysentery | Leaves |
| Diabetes | Roots |
| Nicaragua | |
| Respiratoryandpulmonarydisorders/diarrhea/diuretic/burns | Leaves+seeds |
| Laborpains | Seeds |
| Cough/cold/diuretic/diarrhea/burns/laborpains | Seeds |
| Paraguay | |
| Paraguay | |
| Insecticide/repellent | Seeds |
| Diabetes | Seeds |
| Peru | |
| Aphrodisiac/aphrodisiac/diuretic/antidisenteria/astringent | Fruits |
| Antipyretic/skinproblems | Leaves |
| Alcoholichepatitis/worms | Roots |
| Antipyretic/aphrodisiac/dysentery/astringent/stomach | Seeds |
| TrinidadandTobago | |
| Diuretic | Leaves |
| Diabetes | Roots |
| Diabetes | Roots |

**CONCLUSION**

Bixa orellana has traditionally being used a natural colourant since ancient time by various indigenous community in tropics. It is evident from this investigation that the chemical constituents of plant are responsible for its commercial and medicinal value. Thus there is a need to explore the phyto-therapeutics potential along with application of modern scientific techniques for mass propagation and conservation of this plant.

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