



Bixa Orenalla: A Review on its Phytochemistry, Traditional and Pharmacological uses

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ABSTRACT

Bixa orenalla the sindoor plant or Lipstick Tree, belonging to Bixaceae family has phytochemical and pharmacological use. Being native to the tropical American area, various parts of the plant has a variety of constituents. The bixin and other alkaloids present are being used for different medicinal and cosmeceuticals uses also. Pharmacological activity proved as studied in in-vitro and in-vivo models reports antioxidant, anti-inflammatory, antimalarial, diuretic, hyperglycemic, antidysentric, laxative, as an antidote in snake bite and focusing on treatment and prevention of cancer. It is found safe and effective in various studies performed. Even it is worth noting that most of the works developed with the plant have been produced in tropical America. Special attention should be given by asian scientists to commercialize these historical plant. The published literatures provide information on the origin, chemistry, extraction methods, formulations and toxicological aspects in a quite scattered way. The present review compiles information in the literature that reveals the recent findings on its potential ethanobotanical applications with its Pharmacological and safety aspects.

Keyword: Bixa orenalla, ethanobotanical, toxicological, Bixaceae.



INTRODUCTION

WHO has listed over 21000 plant species used around the world for medicinal purposes.¹ Bixa orellana or Lipstick tree² is one such plant can be found in regions spanning the globe. The plant grows equally well in lowlands and mountainous regions or areas of higher elevation. Native to the tropical American area, B. orellana is found in largest quantities from Mexico³ to Ecuador, Brazil^{3,4}, Paraguay, Colombia, Dominican Republic, Haiti, Trinidad, Mexico, Panama and Bolivia³. It is often cultivated in Madhya Pradesh and South India⁵, Assam, West Bengal³, Orissa, Andhra Pradesh and Maharashtra and to some extent in Kerala, Karnataka, along with Srilanka, Indonesia, East Africa⁴. Plant Bixa Orellana is a dicot plant⁶ belonging to family Bixaceae, genus Bixa⁷, is a dicot Shrub tree perennial⁶ in nature. There are a variety of common names for B. orellana because it flourishes in a variety of places³.

Vernacular Names: It is known as “annato” or “achiot” in North America^{8,3}, “changuarica,” “k’u-zub,” and “pumacua” in Mexico³, “annato” and “urucu” in Brazil³, “urucum” in Germany^{9,3}, “roucou” French West Indies¹⁰, “achiot” in

Colombia¹⁰, “arnotto” in Amerindian¹⁰, Latkan in Hindi, Bengali and Oriya⁵, Ureirom in Manipuri⁵, Rangamali in Kannada⁵, Jabara, Manjitti⁵, Sappiravirai in Tamil², Kurannumannal in Malayalam, Sendri in Marathi², Sinduri in Gujarati², Jolandhar in Assamese². Among the natural dyes annato appears to be the only one, which has great economic and commercial importance³. It is reported to possess various activities like antimicrobial activity¹¹, diuretic¹², antidiabetic and anticonvulsant¹³, analgesic¹⁴, antioxidant¹⁵, antihistamine¹⁶, hypocholesterolemic effect¹⁷, and anticarcinogenic activity¹⁸. Annato dye is a non-toxic natural product, used as colouring agent for various food products. It contains the pigment bixin, which is commonly used in the food and cosmetics industries to add yellow or red colors¹⁹. It was also used for colouring silk and cotton³. Indigenous people still use the pulp for 'cosmetic purposes', as hair dye or lip stick, hence the English common name 'Lipstick tree'. The pulp is also said to repel insects and to protect against sunburn due to the UV-filtering properties of the carotenoid pigment known as Bixin²⁰. The ancient history reveals the whole tree as a valued medicinal plant that has been used to treat a wide variety of conditions from fevers to cancer²⁰.

Historical Uses: Lack Sufficient Evidence like antacid, anticoagulant, apnea, ascaridiasis, asthma, astringent, blisters, blood cleanser, burns cardiotoxic, cataracts, conjunctivitis, cuts, diabetic neuropathy, edema, enhanced immune function, eye infections, fever, food uses, hair tonic, headaches, heartburn, hemorrhage, hemorrhoids, hepatitis, hepatoprotection, jaundice, kidney disorders, liver disorders, measles, mouth and throat inflammation, muscle relaxant, nausea, pleurisy, prostate health, rectal complaints, renal impairment, respiratory distress, sedative, skin disorders, stomach acid reduction, sunstroke, tonic, tonsillitis, urinary retention, uterine disorders, vaginitis, vomiting, weight loss, wound healing¹⁹. The indigenous knowledge and use of *bixa orellana* is referenced in many countries however Brazil is the largest producer of *bixa orellana* seeds. The estimated World Production of Annatto seed in 2006 was found to be 14,500 Metric Tonnes in which 7,000 was used for Domestic consumption and 7,500 was available for export. India produced about 500 Metric Tonnes²¹.

Sindur is a mark of a married woman in Hinduism. The rituals of applying sindur powder in the bride's upper forehead is considered extremely auspicious and has been carried on for centuries. The natural sindoor leads to the avoidance of artificial sindur, which reacts with the head scalp²². Annatto is commonly used as a coloring agent widely used food coloring substances of the food industry²⁰ for pharmaceutical ointments and plasters¹⁹, some people appear to be highly allergic to it and lobby against the use of this additive. Annatto dye is also used to colour hair-oils, shoe polishes, floor polishes, nail-gloss, furniture, brass-lacquer, soap, cosmetic²⁰ and pharmaceutical ointments^{20,19} as well as textiles, wool, leather and calico²⁰.

Variety: The Bixaceae family is one of the smallest plant 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*¹⁶. *Bixa* is often cited by botanists as a monotypic genus represented solely by *B. orellana* L. (syn. *B. purpurea* Hort.) However, a second species was also recognised, *B. arborea* Huber, as a medium to large forest tree of the Amazon region. The *Flora of Peru* (Vol. XIII, Pt. IV, No. 1, 1942) describes the following²³
B. excels, tree, 35 to 100 ft, having spherical, reniform, reddish-brown pods, 1^{3/4}" wide, 1" long, the fragile but thick-based spines breaking off in age.
B. orellana L. shrubby or low, spreading tree, pods ovoid-conical, often pointed, longer than broad, densely spiny, or smooth.
B. orellana L. a small tree with smooth pods.

B. platycarpa, tree to 100 ft., pods are strongly flattened and merely roughened
B. arborea Huber, with regular reniform, strongly compressed pods merely muricate in age.
B. urucurana Willd. Tree to 25 ft.. resembling *B. orellana* but "pods spherical or flattened spherical and smaller, densely to sparsely spiny.
B. orellana L. as the principal species is with two varieties, one having vermilion fruit and the other, yellow fruit being the richer in dye²³.

Description: This plant stands alone in its family as a profusely fruiting shrub that can grow up to about 20 meters².

It is a tropical plant, hence requires warm 32-38°C, dry climate and cannot tolerate wet and foggy climate, and in a wide range of soils from loam to lateritic soils³. The best method of annatto propagation was found budding and stem cutting treated with IAA or IBA⁴. Phosphorous is an important nutrient for Sinduri cultivation⁴. Approximately 50 seeds grow inside of the pod². It yields about 2 kg. of seed for about 20 years³. Although the fruit of the Annatto tree are inedible it is often cultivated for its flowers and more especially for its seedpods²⁰.

Parts used: Seeds, leaves, bark, roots, shoots²⁴

Seeds:

These are in many with a scarlet covering² sized 5mm in diameter¹⁰ and are ovate in shape². Generally Seed maceration or capsules are used. Traditionally it is used as expectorant, ground seed powder in small dosages of 10-20 mg daily is used for high cholesterol and hypertension, insect repellent, wound healer²⁴. In tropical regions seeds are used to soothe an irritated stomach that is suffering from excessively spicy food²⁰.

Leaves

Leaves are large green, 5-15 cm long, 4-11 cm wide and are pointed². Generally the infusion of leaves is used. It is documented to act as aldose reductase inhibitor linked to diabetic complications, antibacterial and antihemorrhagic. Traditionally it is used as antacid, hypocholesterolemic, anti-inflammatory, antiseptic, aperient, aphrodisiac, astringent, digestive stimulant, diuretic, febrifuge, hypotensive, wound healer²⁴. The shoots and young leaves are used for feverish infections including gonorrhoea, dysentery and hepatitis. In tropical regions the leaves are used to soothe an irritated stomach that is suffering from excessively spicy food. They are also said to reduce benign prostate hyperplasia and generally reputed to have anti-tumor activity, which are thought to be due to the high anti-oxidant activity of the

carotenoid compounds Bixin and Norbixin, which are also the source of the red pigment Annatto is known for²⁰.

A 2000 study confirmed the effectiveness of a leaf-and-bark extract at neutralizing hemorrhages in mice injected with snake venom, a practice used in Colombia for many years²⁴. Decoctions of annatto leaves are taken by the half-cupful two or three times daily for prostate and urinary difficulties as well as for high cholesterol and hypertension²⁴.

Flower

They are showy, white or pink in color, nearly 5 cm broad. Flowers occur in vertical upright clusters which prominently appear above the foliage, with five petals and a dense mass of stamens in the center, The petals often tend to curl up with time². An infusion of the flowers are said to be a useful expectorant for new born babies²⁰.

Fruit

They have the fruits which are red, spiny, 2.5-5 cm long and 2-valved and ovoid in shape².

PHYTOCHEMISTRY

Phytochemical analysis of leaf & seed extracts suggested the presence of flavonoids, tannins, saponins, steroids. Alkaloids were detected only in the leaf & anthroquinones in the seeds²⁵. Alkaloids were absent in all extracts of seeds²⁶. In a study the percentage yield obtained from seed extract was highest in Aqueous extract then methanol, ethanol, chloroform, ethylacetate with 13.5, 9.5, 8.5, 3.5, 2 % w/w respectively²⁶ however in leaf extracts, it was highest in methanol, then ethanol, Aqueous, ethylacetate, chloroform with 10.2, 9.5, 7, 2, 1 % w/w respectively²⁷.

Table 1 The table represents various phytochemicals available in the type of solvent extract¹¹.

S.no.	Extract	Seed	Leaves
1.	Aqueous extract	Flavonoids, Steroids, Cardiac Glycosides and Terpenoids ¹¹ , Anthraquinone ²⁶	Alkaloids, Flavonoids, Steroids, Cardiac Glycosides Tannins and Terpenoids ¹¹
2.	Ethanolic extract	Alkaloids, Flavonoids, Terpenoids, and Anthraquinone ¹¹	Flavonoids, Steroids, Cardiac Glycosides and Terpenoids ¹¹ alkaloids, tannins, Triterpenoids, Steroids, Sterols, Saponins, Flavones, Flavonoids ²⁷
3.	Methanolic extract	Flavonoids ¹¹	Flavonoids and Cardiac Glycosides ¹¹ , alkaloids, tannins, Triterpenoids, Steroids, Sterols, Saponins, Flavones, Flavonoids ²⁷
4.	Acetic acid extract	No phytochemicals ¹¹	Alkaloids and Cardiac Glycosides ¹¹
5.	Petroleum ether extract	Tannins ¹¹	No Phytochemicals ¹¹
6.	Dichloro methane extract	--	Sesquiterpene-ishwarano ²⁸
7.	Chloroform extract	Glycosides, Anthraquinone glycosides, Proteins, Tannins, Triterpenoids, Steroids, Saponins Flavonoids ²⁶	Alkaloids, tannins, Triterpenoids, Steroids, Sterols, Saponins, Flavones, Flavonoids ²⁷
8.	Ethyl acetate	Glycosides, Anthraquinone glycosides, Proteins, Tannins, Triterpenoids, Steroids, Saponins Flavonoids ²⁶	Alkaloids, tannins, Triterpenoids, Steroids, Sterols, Saponins, Flavones, Flavonoids ²⁷

Table 2 Chemical constituents present in various parts of plant

S. No.	Plant part	Chemical constituents
1.	Whole plant	carotenoid bixin(80%) ²⁹ and norbixin ³⁰ bixaghanene, bixein, bixol, crocetin, ellagic acid, ishwarane, isobixin, phenylalanine, salicylic acid, threonine, tomentosic acid, and tryptophan ²⁴
2.	Leaves	Maslinic acid, gallic acid, pyrogallol, isoscutelarein ⁵ , flavonoids, heterosides, sulphated derivatives, diterpenes, gallic acid, pyrogallol & essential oils ²⁸
3.	Root	Triterpene, tomentosic acid ^{31,32}
4.	Seed pulp	73 essential oils were identified from seeds, ishwarane and geranylgeraniol as major components in 18.6 and 9.1 % ²⁹ . Carotenoid like Bixin ⁵ , methyl (9Z)-8'-oxo-6, 8'diapocarten-6-oate (2), methyl Z)-10'-oxo-6, 10'diapocaroten-6-oate (4), and methyl (9Z)-14'-oxo-6,14'-diapocaroten-6-oate ¹⁰ , Apo-ψ-carotene, 9'Z-6'-ol Beta carotene, Bixin (red dye) ⁹ , Z-Carotene, Cryptoxanthin, Phytoene, Phytofluene ³³ , Geranylgeraniol ^{10,33} , Lutein lycopenoate, Neurosporene, Norbixin, trans-bixin, Zeaxanthin ³³ , orellin (yellow dye) ⁹ some terpenoids, tocotrienols, arenes and flavonoids including luteolin and apigenin ³¹ (Z,E)-farnesyl acetate, occidentalol acetate, and spathulenol ³⁴

Bixin and norbixin are the principle coloring constituents of annatto. Annatto seeds contain 40-45% cellulose, 3.5-5.5% sucrose, 0.3-0.9% essential oil, 3% fixed oil, 4.5-5.5% pigments (comprised of 70-80% bixin), 13-16% protein, and other constituents³⁵. The concentration of bixin and norbixin recovered after process depends a lot on the type of extraction method and vehicle used³⁶. Bixin is the main constituent of seed coat, shows cytostatic effect on the growth of human lymphoma cells⁵. Bixin was a potent inhibitor of lipid peroxidation³⁵.

In an experiment, the lipid fraction of *B. orellana* seeds was extracted using n-hexane and isolated by thin-layer chromatography showed a higher concentration of delta-tocotrienol¹⁰. The chemical composition of the seed oil of *Bixa orellana* L. by GC/MS identified thirty-five components, of which (Z,E)-farnesyl acetate (11.6%), occidentalol acetate (9.7%), spathulenol (9.6%) and ishwarane (9.1%) were major³⁷.

Annatto oil is rich in bixin¹⁰, tocotrienols, beta-carotene, essential oil, saturated and unsaturated fatty acids, flavonoids and vitamin C. It is easily incorporated into creams, sunscreen, and lip balms where it helps to condition hair and protect against ultraviolet rays⁷. Total phenolic content on spectrophotometric determination of various seeds extract of chloroform, methanol, ethanol and water using maceration measured at 760 nm wavelength showed presence of phenolic compounds in all the extracts but the highest total phenolic content was

observed in aqueous extract of seeds of *B. orellana*³⁸.

SIDE EFFECTS

Annatto is likely safe for most people when used in food amounts. Special precautions include diabetes, as it might increase or decrease blood sugar levels, blood sugar should be monitored and then used. In case of surgery, it might effect by blood sugar levels as it interfere with sugar level during and after surgery³⁹. Pregnancy and breast feeding are the two main parts where no reliable information is gained and so it should be avoided during the time^{39,19}.

TRADITIONAL USES

The tree was incorporated into the traditional medicine of India, where different parts of the plant are used as diuretic, laxative, antibilious, antiemetic, and astringent agents, as a blood purifier, in jaundice, in dysentery⁷, diabetes, diarrrohea, fever, fluid retension³⁹, in peru for heart burn^{39,40}, malaria, hepatitis, antioxidant and bowel cleanser³⁹ for the treatment of wounds and to treat diarrhoea and asthma³⁶, used in the treatment of snake bite as an ingredient in weight- loss products, diabetes and obesity, tonsils, baldness⁴¹ and externally as scar-preventive⁷, vaginal infections⁴⁰, to treat burns, and as insect repellent³⁹.

In developing countries it is also used as folk medicine in the form of decoctions, teas & juices for the treatment of common infections. In Philippines, the leaf decoction is used to cure skin

diseases and burns. The leaves are a popular febrifuge in Cambodia. The infusion of leaves is prescribed as a purgative and in the treatment of dysentery. In Central America, the oil derived from seeds is used to cure leprosy and decoction is given to treat jaundice²⁵. In Jamaica seeds are used for diabetes, and in Peru different parts have reported different activity like fruit for aphrodisiac, diuretic, astringent and leave for skin problems and antipyretic. Roots for alcoholic hepatitis and worms whereas seeds for dysentery astringent and stomach use⁹. Seeds and latex are used for tumors as Seeds gargled with vinegar and rice water for cancer of the mouth. Leaf infusion used in Costa Rica to prevent baldness. Leaf infusion gargled for tonsillitis. Bolivians press leaves on aching body parts. Astringent febrifugal fruit pulp is used for dysentery and kidney disease. The reddish paste is applied as an unguent to burns⁴¹. Local people in Bangladesh use the leaves for a range of illnesses including diarrhoea, sleeplessness and skin diseases³¹.

A tea made with the young shoots is used by the Piura tribe of Rainforests of Amazon as an aphrodisiac and astringent and to treat skin problems, fevers, dysentery, and hepatitis. The leaves are used to treat skin problems, liver disease, and hepatitis. The Cojedes tribe uses an infusion of the flowers to stimulate the bowels and aid in elimination as well as to avoid phlegm in newborn babies. Traditional healers in Colombia have also used annatto as an antivenin for snakebites. The seeds are believed to be an expectorant, while the roots are thought to be a digestive aid and cough suppressant.

Leaf decoctions are used to treat heartburn and stomach distress, as a mild diuretic and mild laxative and to treat burns in Brazilian herbal medicine and also in Peruvian herbal medicine to treat prostate disorders and internal inflammation, arterial hypertension, high cholesterol, cystitis, obesity, renal insufficiency, and to eliminate uric acid⁴².

For epilepsy, the juice is used in a combination of 12 fruits and taken twice daily for 5 days. In Peru, *Bixa orellana* leaf was used to treat mild indigestion and to strengthen the liver. A tea made with the young shoots was used as an aphrodisiac and astringent, and to treat skin problems, fevers, dysentery, and hepatitis. In Suriname, *Bixa orellana* tea was drunk to treat nausea. The root is believed to have Cough Suppressant activity¹⁹.

An infusion of the leaves and roots is useful in epilepsy, dysentery, fever and jaundice³¹. Decoction from leaves and seeds are used to stop

vomiting and nausea, treat heartburn, prostate and urinary difficulties, stomach problems⁴³, treat diabetes, leaves infusions are used in external applications in the case of chickenpox and leprosy. It is used as urinary and vaginal antiseptic⁴⁴.

This data shows that several of the popular uses of *Bixa orellana* are the same, for example, antipyretic, aphrodisiac, antidiarrheal, antidiabetic, and insect repellent in the American countries

In India, leaves are used for jaundice and snakebite, the root bark for fevers, including malaria. Fruits are considered astringent and laxative. The plant is also recommended for gonorrhoea. The hot water extracts potently inhibit lens aldose reductase, perhaps due to isoscutellarein. The foliage of *Bixa* is used to treat skin problems and hepatitis, also used as aphrodisiac, antidysenteric, and antipyretic. It is considered good for the digestive system and for treatment of liver disease. But all these uses have no scientific confirmation. Research is going on to explore these uses of *Bixa*⁴¹.

Ayurveda practitioners in India use it as an astringent and mild purgative and are considered as a good remedy for treating dysentery and kidney diseases. The root bark is anti-parasitic and antipyretic. The traditional healers claim that *Bixa* species are more efficient to treat infectious diseases than synthetic antibiotics²⁵. The preparations involve powder of seed, pulp and decoction⁴⁵.

Bixa orellana products are often marketed as herbal treatments for liver conditions, urinary conditions, heartburn, digestive and prostate problems, internal inflammation, arterial hypertension, high cholesterol, cystitis, obesity, and renal insufficiency, and to strengthen the immune system. *Bixa orellana* is also used in multi-ingredient weight-loss products¹⁹.

PHARMACOLOGICAL ACTIVITIES

According to dr dukes data the Ethnobotanical uses of *Bixa orellana* includes Ache, Antidote, Aphrodisiac, Astringent, Burn, Cancer, Coloring, Cosmetic, Diabetes, Diuretic, Douche, Dysentery, Epilepsy, Excitant, Fainting, Fever, Flu, Gonorrhoea, Hair-Oil, Inflammation, Jaundice, Malaria, Parasiticide, Purgative, Refrigerant, Insect Repellent, Stomachic, Stomatitis, and in Throat infection⁵⁴. Along with this it is also reported to have antioxidant, broad antimicrobial (antibacterial and antifungal), anti-inflammatory, analgesic, hypoglycemic, and antidiarrheal activities⁵⁵

Antimicrobial activity: The antibacterial activity of ethanolic, methanolic, acetone and dimethyl

sulphoxide extracts of *Bixa orellana* leaves and deseeded fruit capsule were studied against *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus* *Staphylococcus aureus* by disc diffusion method and it possessed significant activity³⁶. Along with this an invitro study on methanolic extracts of the leaf & seed of *Bixa orellana* L. showed significant inhibition against strains of *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Vibrio cholera*, *Moraxella catarrhalis*, *Acinetobacter* species, *Brucella* species, along with fungal pathogens *Candida albicans*, *Aspergillus niger* and the dermatophytes *Trichophyton mentagrophytes* & *Trichophyton rubrum*²⁵. On a study of callus derived from different explants, for antimicrobial activity, the leaf callus showed maximum activity against *Bacillus pumilus*⁵¹. Ishwarane the essential oil is responsible for exhibiting moderate antifungal activity against *Candida albicans*³².

Hypocholesterolemic Activity: The aqueous extract of seeds of *Bixa orellana* exhibit a hypocholesterolemic effect without causing any liver injury as shown in the study of Male Fisher rats fed with the high-lipid diet lowered the LDL- and total cholesterol and raised the HDL-cholesterol, which is the lowering effect on lipid profile without any significant effect on serum levels of albumin or serum activities of transaminases¹⁷.

Antileishmanial: Leishmaniasis is a disease caused by *Leishmania* protozoa. There is currently no vaccine against leishmaniasis, and the in vitro and in vivo effects of the essential oil from *Bixa orellana* seeds against *Leishmania amazonensis* were evaluated which showed the ability of *Bixa* oil to control disease progression of established cutaneous leishmaniasis in mice after a treatment with 30mg/kg by intraperitoneal administration over 14days⁵³. Geranylgeraniol, one of the main component of the oil isolated from its seeds showed activity against the intracellular amastigote form of *Leishmania amazonensis*³².

Antioxidant & a natural food preservative: The ethanolic extract from *Bixa orellana* leaves has potentials to be used as a food preserver (antimicrobial and antioxidant agent). The antiradical properties combined with the bactericidal and fungicidal activities increases the potential application of ethanolic extract as a natural food preserver offered with its low toxicity²⁸. In vitro assay of *Bixa orellana* seed extract using DPPH and Ferric iron reducing power models also showed antioxidant activity at low

concentrations¹⁵. Antioxidant activity is also in relation to CNS disorders, as the metabolism of phospholipids is associated with neuronal death in Alzheimer's disease⁵⁶ and antioxidants such as Vitamin E play an important role in amyloid aggregation³¹.

Antidiarrhoeal Activity: *Bixa orellana* possesses antidiarrhoeal activity as in the study using castor oil-induced diarrhoea model and gastrointestinal motility test in mice. Castor oil causes diarrhoea through its active metabolite ricinoleic acid, which stimulates the peristaltic activity of small intestine leading to changes in electrolyte permeability of intestinal mucosa. Its action is also associated with stimulation of release of endogenous prostaglandins³¹.

Antidiabetic: Methanolic extract of *Bixa orellana* seed showed significant hypoglycemic activities when administered 15 min. after glucose load using a modified oral glucose tolerance test with swiss webster mice as a test animal. An infusion of *B. Orellana* was found to lower blood glucose level when administered 45 min before glucose load⁴¹. Antidiabetic activity on streptozotocin induced diabetes on male wistar rats also showed hypoglycemic effect of annatto lasting for twelve hours of evaluation. This effect was small and did not prevent the weight loss that is characteristic of decompensated diabetes mellitus²⁹. *Bixa orellana* is believed to lower blood glucose by stimulating peripheral utilization of glucose, on C-peptide and streptozotocin-induced diabetic dogs. The extract was found to decrease blood glucose levels in fasting normoglycaemic and streptozotocin-induced diabetic dogs⁵⁷. However in another antidiabetic activity study better activity was recorded from the extracts of ethyl acetate and n-butanol, when studied in comparison to other extracts like ethanol, petroleum ether, diethyl ether¹³.

Hepatoprotection: Methanolic extract of *B. orellana* seeds illustrated hepatoprotective activity against Swiss albino rats with liver damage induced by carbon tetrachloride (CCl₄). Blood samples of CCl₄ treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by it and blood samples from the animals treated with the methanol extracts of *B. orellana*, showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells⁴².

Antihistamine: The aqueous extract of *B. orellana* suppresses the histamine-induced inflammation in rats as exposure of rat's paw to histamine triggered an elevation in fluid extravasations from

microvessels in the vicinity leading to tissue swelling, but the *Bixa orellana* extract leads to the suppression of increased vascular permeability reflected in the decreased paw volume¹⁶.

Cardioprotective Activity: The ethanolic extract of plant possess Cardioprotective activity against isoproterenol-induced myocardial infarction in Swiss albino rats, as per the evaluation of protective effect through measuring the levels of Cardiac marker enzymes, uric acid, cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and high density lipoprotein⁴⁹.

Diuretic Activity: The plant posses diuretic activity and in the research it was reported to show significant effect in methanolic extract as compared to the petroleum ether and aqueous extracts of leaves. The result was evaluated by measuring the total volume of urine and levels of Na⁺, K⁺ and cl⁻ in urine¹².

Neuropharmacological Activity: *Bixa orellana* leaves extract when monitored through pentobarbitone-induced hypnosis, open-field and hole-cross tests showed a depressing effect on the CNS and a decrease in locomotor activity which leads to the reduction in time for the onset of sleep

and increase in the duration of total sleeping time³¹. However diethyl ether extract of the plant *Bixa orellana* have better anticonvulsant activity¹³.

Analgesic Activity: Leaf extracts are useful in headaches, reported in the investigation for analgesic activity using the acetic acid-induced writhing model. The response was not exactly defined but the analgesic activity reported with the reason due to stem from its ability to interfere with the synthesis and/or release of those endogenous substances or desensitization of the nerve fibers involved in the pain transmission pathway³¹.

Antimalarial: In the isolation and structural elucidation of the major extract constituents of *B. orellana* hairy root culture was done, where stigmasterol was identified as the most potent compound lead, the other chemical constituents of *B. orellana* hairy roots showed modest antimalarial properties with low toxicity in mammalian cells³⁴.

Antivenom activity: The neutralization of the hemorrhagic effect of the venom of *Bothrops atrox*, was tested in *Bixa orellana* and it was found 100% effective. It also has a neutralizing activity against the edema forming, defibrinating and coagulant effects of *B. asper* venom³².

Table 3 Pharmacological Activities of the plant

S. No.	Plant part	Uses
1.	Plant	Anticlastogenic activity ³¹ , Astringent, antibilious, antiemetic, blood purifier ⁵ , leprosy, eczema, elephantitis ^{46,47} , dysentery, gonorrhoea, fever ⁴⁵ , Neurological, anticonvulsant, analgesic, antidiarrhoeal, diuretic activity, diabetes mellitus, antiperiodic and antipyretic. Activity against protozoan ¹⁴ , helminthes and has platelet antiaggregant activity ^{48,11} . As treatment for gastric ulcers and stomach discomfort ¹⁶ , Cardioprotective ⁴⁹ , Spasmolytic ³¹
2.	Leaves	Antimicrobial, infusion given in jaundice, dysentery, externally scar preventive ⁵ also in bronchitis, sore throat and eye inflammation ⁹ , antifungal, antileishmanial, anticonvulsant, analgesic and antiinflammatory ¹⁶ , diuretic ¹² , Epilepsy and fever ¹¹ along with Snake antivenom properties ⁵⁰ . The decoction of leaves is used in antiemetic therapy during pregnancy ^{32,9} , gonorrhoea, liver diseases ⁵¹ , taking Laxative, oral tumours ²⁸ . It is also used in brazil for heart burn and indigestion ¹⁹ .
3.	Stem	Snake antivenom properties ¹¹ fresh shoots steeped in water as an eye wash for inflamed eyes ³²
4.	Root	Epilepsy, dysentery ⁵² , fever and jaundice ¹¹ , diabetes and hypertension ¹⁶ , for treating sore throats, gonorrhoea, liver diseases ⁵¹ , smooth muscle-relaxant activity in guinea pigs, lowered gastric secretions in rats, digestive aid ²⁴ , Antiflu and against tuberculosis ⁴⁴ , diuretic ³²
5.	Root bark	Febrifuge, antiperiodic ⁵ , Gonorrhoea treatment ¹¹
6.	Seed pulp	Hyperglycemic, Antidysentric, diuretic, haemostatic, laxative ⁵ , purgative, anti-pruritic and for buccal tumors ³⁶ , Gonorrhoea treatment ¹¹ , Expectorant, Hypotensive and Antibiotic ⁹ , Hypocholesterolemic effect ¹⁷ , Antileishmanial ⁵³ , Analgesic ⁴¹ , anti-inflammatory ²⁹ , chemopreventive ³¹ and antioxidant activity ^{15, 31} . The seed paste is indicated as an aphrodisiac and as protection against insect stings and in syrup against pharyngitis and bronchitis. seeds are also recommended in cases of intestinal catarrh, measles and as an emmenagogue ³² , astringent ⁴¹
7.	Fruit	Antidysentric ⁵

TOXICOLOGICAL ACTIVITIES

Safety issues arise whenever medical choices have to be made⁵⁸. Anything added to food that is natural or, in other words, grown from seed is not under careful scrutiny by the Food and Drug Administration. Annatto, which is generally safe to eat, comes under this category. In a study regarded irritable bowel syndrome, it was observed that it is not at all a significant producer of allergic responses⁴⁰.

The plant has no cytotoxic effects as reported by the toxicity study in mice with methanolic extract from *B. orellana* leaves and seeds at doses upto 4000mg/kg²⁸.

The plant was also evaluated for its mutagenicity and antimutagenicity activity in various concentrations of 1330, 5330 and 10,670 ppm, performed in bone marrow cells from Swiss male mice, and the data indicated that annatto colour, is neither mutagenic nor an inhibitor of induced mutations, although it should be used carefully since high doses may increase the effect of a mutagen⁵⁹. However a 6 month study in humans given 750 mg of leaf powder per day demonstrated no significant or serious adverse effects⁵⁵. It is likely safe to be used orally in food amounts in nonsensitive individuals and Possibly safe when used medicinally in small dosages of 10–20 mg of powdered *Bixa orellana* leaf tablet daily for up to 2 weeks, based on expert opinion and animal study.

It is possibly unsafe to be used in patients with bleeding disorders or those using anticoagulants, with impaired kidney function, taking diuretics, antihypertensive agents, taking mutagenic agents as cyclophosphamide, using cytochrome P450 substrates, with a history of constipation or those who are using laxative, antidiabetic agents, or using CNS depressants. Annatto may lower blood pressure, urticaria and angioedema. Eczema may also occur. Annatto may alter glycemic and insulin levels¹⁹.

Other Applications

Bixa orellana is widely used for obtaining coloring agents that has applications in foods, body paints, arts, crafts and murals¹¹. The lower cost of production and the low toxicity, make annatto a very attractive and convenient pigment in substitution to the many synthetic colorants. Annatto is found neither genotoxic nor carcinogenic at the highest concentration tested (1000 ppm) hence is extensively used as a natural food colorant¹⁸. The dye obtained from the pulp of the *B. orellana* seed (bixin) is used all over the world as a red-orange dye for coloring rice¹⁰,

cheeses^{10,40}, soft drinks, oil, butter, soup¹⁰, margarine, mayonnaise, sauces, mustard, sausage, soup, juice, ice cream, bakery products, macaroni¹⁰, in crackers, cereals, food dressings, ice creams, cookies, etc. Hence, it is frequently ingested⁴⁰.

The extract of the seeds wards off insects and protects the skin against the ultraviolet rays of the sun. It is also used to colour hair-oils, shoe polishes, floor polishes, nail-gloss, furniture, brass-lacquar, soap, cosmetics and pharmaceutical ointments as well as textiles, wool, leather and calico²⁰. *B. orellana* seeds were used to dye local made bathing towels known as 'phadi'⁶⁰.

The coloring and flavoring substances from the aril can also be extracted by heating the seeds in oil or lard, which is then used in cooking. These condiments are used on many dishes and processed foods¹¹, such as cheese^{61,11,7}, butter, soups, gravies and sauces, cured meats¹¹, margarine, chocolate, fabric and paints⁷, soft drinks, sugar confectionery, ice cream, fish products⁶², and many more. In food as coloring agent³⁹ mainly to impart a yellow to reddish-orange color to the food, but to some extent also for their subtle flavor and aroma. They can be used to color and flavor rice instead of the much more expensive saffron. In preparations like orange squash, synthetic syrup, mango pulp, tomato ketchup¹¹.

The dye exists under the list of permitted colors in accordance with the rule 28 of Prevention of Food Adulteration Act (PFA) and also specified in Food Chemical Codex of USA. The colour is 'Generally Recommended as Safe (GRAS)' for human consumption in usage of food products⁶³.

EXHAUSTED SEED COAT

Pigment extracted annatto seed contains the crude protein content 14.35% and a crude fibre content 25.60%. The mineral analysis revealed that it has a high phosphorous content and a low calcium content. The chemical composition in the pigment extracted annatto seed in the (%) value was found Dry matter 86.81 (%), Organic matter 94.77 (%), Total ash 5.23 (%), Nitrogen free extract 52.87 (%). The fibre fraction contains hemicellulose, cellulose, silica and lignin contents as 34.0, 14.4, 0.30 and 10.86 %, respectively. The calcium, phosphorous, magnesium, potassium and sulphur contents were 0.04, 0.7, 0.2, 1.1 and 0.35 %, and the iron, manganese, cobalt, zinc and molybdenum contents were 142, 2125, 2.07, 132 and 203 ppm, respectively. Hence it was concluded to be used as substitute for cereal grains in the rations of buffaloes as a source of energy and by-pass protein⁶⁴.

CONCLUSION

Looking upon wide prospects and potentials of plant for various purposes, it is needed to be widely cultivated in most of the areas where climatic conditions favor its optimum growth. In this way, maximum yield of its different useable parts could be achieved to derive the maximal amount of commodities of a multifarious nature for the welfare of mankind. So far numerous studies have

been conducted on different parts of plants in countries other than India and the data shows that several of the popular uses of *Bixa orellana* are the same, for example, antipyretic, aphrodisiac, antidiarrheal, antidiabetic, and insect repellent in the American countries, but systematic and scientific research in Indian heritage is required to explore the maximum potentials which are needed to be isolated and identified from different parts of the tree, for human and environmental well being.

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