

Ethnobotany, Phytochemistry, and Pharmacological Efficacy of *Bixa orellana*: A Review

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Abstract

Bixa Orellana, commonly known as lipstick tree, is a small perennial tree with reddish triangular fruits. It is commonly utilised as a natural dye, which found a wide range of applications in both foods and textile industries. It is also used in traditional medicinal practices to treating various health ailments. This review aims to summarise the ethnobotanical, phytochemistry as well as the pharmacological potential of *Bixa Orellana*. It has been used for fever, skin problems, intestinal problems, and hepatoprotection by the various tribal communities worldwide. It is also employed as an antidote to bites and as an insect repellent. The phytochemical review demonstrated numerous bioactive compounds such as alkaloids, tannins, terpenoids, saponins, flavonoids, etc. The pharmacological investigations evidenced that the plant has excellent antimicrobial, antipyretic, antidiabetic, anticonvulsant, antioxidant, and anticancer properties. In addition, *Bixa Orellana* can be used as a photosensitizer in antimicrobial photodynamic inactivation therapy to eliminate micro-organisms. So, the pieces of evidence highlighted in this review will be helpful insights for further scientific studies for the discovery and development of novel therapeutic agents.

Keywords: *Bixa orellana*, Annatto, Ethnobotanical, Pharmacological activity, Photosensitizer, Photodynamic inactivation.

Introduction

The plants and trees are considered as the natural repository for the numerous bioactive compounds. So it has diverse applications in pharmaceutical, food, and cosmetic industries from the olden days (Patra et al., 2018). The plants used in traditional medicinal practices gains much attention among researchers for the development of new products and drugs (yuan et al., 2016).

One such traditionally medicinal plant is *Bixa Orellana*, a well-known natural dye-yielding plant habitually used by some ethnic groups (Mahanta et al., 2005). It belongs to the family Bixaceae, and it is commonly known as annatto, achiote, or sometimes as lipstick tree. It is a shrub or small perennial tree that ranges about 3-10m with pink or white flowers, reddish-brown fruits, and reddish triangular seeds (Aher et al., 2012; Umadevi et al., 2020). It is commercially employed as a natural food colourant in the food industries to replace synthetic dyes, and the seeds are used as a condiment (Leal and Clavijo 2010). Some of the bioactive constituents isolated from *Bixa Orellana* seeds

are used for making some dietary products (Quintero et al., 2020). It is native to tropical regions and grows well in alluvial soil with pH ranges from 6 - 7.5. The economic life span of *Bixa Orellana* is 20-25 years. The ethnobotanical studies of *Bixa Orellana* have revealed its therapeutic significance for treating various diseases, and so it has been used as a traditional medicine throughout the world (Venugopalan et al., 2011). So, in this review, we have summarised the ethnobotany, phytochemistry as well as pharmacological activities of *Bixa Orellana*.

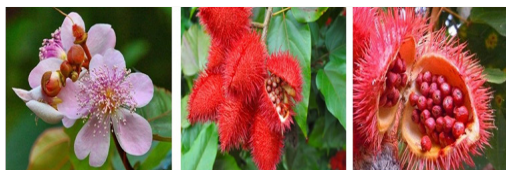


Figure 1: Flower, Fruits, and Seeds of *Bixa Orellana*

Ethnobotanical Uses

Nature has been blessed with enormous plant species with a wide range of applications. But most of the knowledge about the plants and trees are well-known by the indigenous people. The tribes

usually depend on natural resources like plants and trees for treating diseases and also their day-to-day activities (Purushothaman and Irfana, 2020). Ethnobotanical studies of medicinal plants should be documented properly to conserve indigenous knowledge (Ezhilvalavan et al., 2015). The tribal people have been using *Bixa Orellana* for treating various diseases and also as a natural dye. Here, we have documented some of the ethnobotanical uses of *Bixa Orellana* by the various tribal communities worldwide.

Botanical Classification

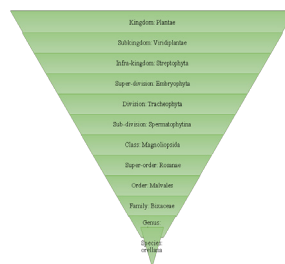


Figure 2: Botanical Classification of *Bixa Orellana*

People	Native	Parts Used/ Mode of Preparation	Uses	Reference
Amazon tribe	Brazil	<ul style="list-style-type: none"> • Macerated seeds • Infusion made from roots • Seeds 	<ul style="list-style-type: none"> • Contusion • Vaginal discharge • uterine problems • Body painting 	Coelho-Ferreira, 2009
Assamese tribe	Assam, India	Dried seed powder mixed well with slaked lime	Dyeing cotton and silk garments	Kar and Borthakur, 2008
Bagata tribes	Andhra Pradesh, India	Oral administration of root extract twice for 2 days	Fever	Sri and seetharami, 2019
BaigaandGond Tribe	Chhattisgarh, India	Seeds	Skin diseases and Intestinal problems	Soni, 2013
Bedes tribes	Bangladesh	Leaf paste	Applied on injured or painful region twice regularly for 7 days	Seraj et al., 2013
Brazilian tribes	Brazil	Roots, leaves, and seeds	Epilepsy	Potraj et al., 2019
Chothe tribe	Manipur, India	Bark, leaves, and seeds	Antidote for snake bite, astringent, antipyretic	Sanglakpam et al., 2012
Cojedes tribe	South america	Infusion made from flowers	Reduce mucus production in infants	Ulbricht et al., 2012
Koyas and kondaReddis tribes	Andhra Pradesh, India	Administration of seed pulp twice a day	Dysentery and diarrhoea	Raju and Reddy, 2005

Khonds tribe	Andhra Pradesh, India	Buds and flowers	Food colorants	Rao et al., 2006
Jagdapur district tribes	Chattisgarh, India	Leaves, seeds, and roots	Malaria, urinary infections, and jaundice	Bargali et al., 2003
Meitei and Rongmei tribes	Manipur, India	Seeds soaked in water to get red dye	Dyeing yarn threads and clothes	Kikim et al., 2015
Piura tribe	Amazon Rainforest	Tea made from young roots	Skin related problems, aphrodisiac, and astringent	Raddatz-Mota et al., 2017
Tonchongya tribes	Bangladesh	Topical application of powdered whole plant on painful area	Breast pain	Jahan et al., 2015
Wajapi tribe	Brazil	Seeds	Insect repellent	da Mata et al., 2012

Phytochemical Studies

A broad range of phytochemical constituents is present in the *Bixa Orellana*, evidenced for its ethnobotanical uses. The phytochemical analysis carried out with aqueous alkaline extract of *Bixa Orellana* seeds revealed tannins, terpenoids, saponins, carbohydrates, glycosides, and flavonoids (Abayomi et al., 2014). The study was done by Tamil Selvi et al., 2011, suggested the presence of phytochemicals such as flavonoids, tannins, saponins, and steroids in the methanolic extract of *Bixa Orellana* seeds and leaves. But, the alkaloids were present in leaf extract, whereas the seed extract contains anthraquinones. The phytoconstituents such as carbohydrates, proteins, amino acids, steroids, glycosides, tannins, phenols, saponins, flavonoids, and alkaloids were present in the aqueous, alcoholic, and hydroalcoholic of *Bixa Orellana* seeds and leaves (Sangvikar et al., 2015). The preliminary phytochemical studies revealed Alkaloids, Flavonoids, Tannins, Terpenoids, Saponins, glycosides, and Steroids in the ethanol extracts of *Bixa Orellana* bark (Panda et al., 2018). The GCMS analysis with ethanol seed extract of *Bixa Orellana* resulted in bioactive constituents such as Haptadecanoic acid, Benzene, Acetic acid, Octadecadien-1-of-Octadecanoic acid, 2,4-Imidazolidinedione, Phthalic acid, and allyl ethyl ester (Nagamani et al., 2015).

Anti-Microbial Activity

The ethanolic, methanolic, acetone and dimethyl sulphoxide extracts of *Bixa Orellana* (leaves and deseeded fruit capsule) exhibited antibacterial

activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus* at the concentration of 1600µg (Venugopalan and Parvatam, 2012). The annatto extract (pigment of *Bixa Orellana*) showed potential bactericidal activity by inhibiting the growth of *Bacillus cereus*, *Clostridium perfringens*, *Streptococcus thermophilus*, *Lactobacillus casei* subsp. *Casey*, *Lactococcus lactis*, *Paenibacillus polymyxa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus durans* (Galindo-Cuspinera et al., 2003). The ethanol extract of *Bixa Orellana* leaves, roots, and hypocotyls were found to have remarkable inhibitory effects on *Bacillus pumilus* (Castello et al., 2002). The lyophilised hydroalcoholic extracts of Stem, root, and leaf of *Bixa Orellana* exhibited bactericidal activity against *Proteus mirabilis* (ATCC 25933), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 12228), and *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella typhimurium* (ATCC 14028). Interestingly, these extracts also exhibited remarkable antimycobacterial activity on *Mycobacterium tuberculosis* (ATCC 25177) (Silva et al., 2010).

The methanol extract of *Bixa Orellana* leaves and seeds showed anti-fungal activity against the *Candida albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* (Tamil Selvi et al., 2011). Similarly, the *Bixa Orellana* leaves ethanol extract also inhibited the growth of *Candida albicans* (Poma-Castillo et al., 2019). The methanolic extract of young leaves of *Bixa Orellana* inhibited the growth of dermatophytes such as *Candida*

albicans, *Microsporium gypseum*, *Trichophyton tonsurans*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* (Singh and Vidyasagar, 2017).

Anti-Diarrhoeal Activity

Bixa Orellana leaves extract has demonstrated its significant anti-diarrhoeal activity by reducing the total number of faeces, including wet stools, in the castor oil-induced diarrhoea mice model (Shilpi et al., 2006). Administration of 50, 100, and 200 mg/kg of hydroethanolic extract of Bixa Orellana leaves exhibited effective anti-diarrhoeal activity in castor oil-induced diarrhoea in the experimental mice model (Fokam Tagne et al., 2019).

Analgesic Activity

The acid-induced writhing test of Bixa Orellana leaves reduced the writhing reflex ($p < 0.001$), thereby proving its potent analgesic activity (Shilpi et al., 2006). The analgesic property evaluated by the tail immersion method demonstrated that the methanolic extract of Bixa Orellana leaves possesses maximum activity at the dose of 500 mg/kg (Radhika et al., 2017). Interestingly, the methanol extract of Bixa Orellana leaves manifested remarkable analgesic activity in acetic acid-induced writhing, hot plate, formalin-induced nociceptor, and tail immersion test. In addition, it also revealed significant neuropharmacological activity in a dose-dependent manner (Aktary et al., 2020).

Anti-Histamine Activity

The oral administration of aqueous extract of Bixa Orellana leaves inhibited (60.25%) histamine-induced paw oedema in male Sprague–Dawley rats at the concentration of 150 mg/kg (Yong et al., 2013a).

Anti-Parasitic Activity

The evaluation of the anti-parasitic activity of hydroethanolic extracts of Bixa Orellana stem, leaves, and roots against *Leishmania amazonensis* (M2269) was appeared to be effective at the concentration of 0.21 mg/mL, 0.14 mg/ml, and 0.44 mg/mL, respectively. These results supported the anti-parasitic claims of Bixa Orellana (Rodrigues et al., 2013). Additionally, the study done by García

et al., 2011 revealed the anti-parasitic activity of ethanolic extract of Bixa Orellana seeds against promastigote and amastigote forms *Leishmania amazonensis*. The intraperitoneal administration of 30mg/kg of essential oil from Bixa Orellana seeds inhibited the disease progression in *Leishmaniasis* induced BALB/c mice model (Monzote et al., 2014).

Anti-Inflammatory Activity

Aqueous Leaf extracts of Bixa Orellana exhibited potential anti-inflammatory effects on the carrageenan-induced plantar oedema in the Wistar rat model, which was comparable to the indomethacin (positive control group) (Zarza-García et al., 2017). The anti-inflammatory of crude aqueous extract of Bixa Orellana leaves reduced nitric oxide production in the bradykinin-induced hind paw oedema model at the dosage of 50 and 150 mg/kg (Keong et al., 2011). The work was done by suggested anti-inflammatory activity by suppressing the paw volume and serotonin-induced acute inflammation in the air pouch model (Yong et al., 2013b). The petroleum ether extract of Bixa Orellana leaves exhibited potent anti-inflammatory at the concentration of 250 mg/kg (Radhika et al., 2017).

Anti-Convulsant Activity

The oral administration of ethanol extract of Bixa Orellana bark at the dose of 200 mg/kg was found to have protector activity pentylenetetrazole (PTZ) induced seizures in the male mice model. Consequently, it also reduced the hind limb tonic extensions (HLTE) induced by Maximal electroshock (Panda et al., 2018). The diethyl ether extract of Bixa Orellana leaves exhibited an anticonvulsant effect more than the standard drug Clonazepam in the strychnine administrated Swiss albino mice model (Patnaik et al., 2011).

Anti-Diabetic Activity

The ethyl acetate and butanol fraction of Bixa Orellana leaves exhibited potent anti-diabetic activity in the alloxan-induced diabetic mice model (Patnaik et al., 2011). The Bixa Orellana (annatto) pigment revealed its antidiabetic property by lowering the blood glucose level in the streptozotocin-induced diabetic mice (Teles et al., 2014). The work done

by Ponnusamy et al., 2011., also suggested the hypoglycemic property of Bixa Orellana methanol extract via inhibiting the Human Pancreatic Amylase with the IC₅₀= 49 µg/mL-1.

Antioxidant Activity

The antioxidant activity evaluated using the DPPH method showed scavenging activity of 92.47%, 92.47%, and 91.72% by aqueous extract, ethanolic extract, and methanolic extract of Bixa Orellana leaves respectively, at the concentration of 800 µg/mL (Ahmed et al., 2020). The acetone, chloroform, ether, and methanol extracts prepared with leaves of Bixa Orellana manifested their notable antioxidant properties in TLC-based DPPH assay (Bhatnagar et al., 2015). The bioactive compound bixin and its derivative nor-bixin found in the Bixa Orellana seeds extract were appeared to be an excellent antioxidant agent (Nathan et al., 2019). Aqueous extract of Bixa Orellana bark at the dosage of 250 mg/kg decreased the level of alanine aminotransferase ($p < 0.001$) as well as malondialdehyde ($p < 0.001$) and thereby proving its antioxidant property (Djibersou et al., 2020).

Anticancer Activity

Petroleum ether extract of Bixa Orellana evaluated against B16F-10 melanoma cell line demonstrated its strong anticancer and apoptosis-inducing capability (Kumar and Latha, 2016). The tocotrienols found in the Bixa Orellana seeds possess higher anticancer activity against malignant human A375 melanoma cells (Beretta et al., 2018).

Diuretic Activity

Methanolic leaves extract of Bixa Orellana increases the total volume of urine in Wistar rats at the concentration of 500 mg/kg body weight, which proved its excellent diuretic activity. Interestingly, leaves extract also increases the level of potassium, sodium, and chloride in urine than the standard drugs Furosemide and Arachis oil (Radhika et al., 2010).

Antimicrobial Photodynamic Activity

Antimicrobial photodynamic inactivation is an approach to eradicate microbes with the help of photosensitisers (Purushothaman and Irfana, 2021). Hence, the reddish pigment annatto in Bixa Orellana can act as a photosensitiser, which works with LED

to provoke antimicrobial photodynamic inactivation (Gonçalves et al., 2018). The antimicrobial photodynamic inactivation therapy carried out with Bixa Orellana extracts using blue light-emitting diode demonstrated the immediate suppression of Halitosis (Gonçalves et al., 2020).

Conclusion

This review has highlighted some of the ethnobotanical uses and pharmacological properties of Bixa Orellana. It has become one of the notable substitutes for synthetic dyes, but it also has significant pharmacological properties, which ultimately aids in the novel drug discovery. Some scientific studies manifested the remarkable bioactivity such as antimicrobial, anti-diabetic, anti-analgesic, anti-cancer, anti-inflammatory, and antioxidant properties of Bixa Orellana extracts. Although, more researches are still needed to evaluate the traditional and ethnopharmacological properties of Bixa Orellana. This review also shed light on the potency of Bixa Orellana as a photosensitiser for eliminating pathogens via antimicrobial photodynamic inactivation. Many scientific studies were carried out on the leaves and seed extract of Bixa Orellana. But, all the parts of Bixa Orellana has been traditionally used to treat various diseases. So each part of this tree needs to be explored to find out the novel therapeutic activity.

Authors Contributions

The authors contributed equally for concept making, data acquiring, investigating, and writing the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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