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A DETAILED REVIEW OF THE PHARMACOGNOSTICAL STUDY OF *BIXA ORELLANA* L.

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ABSTRACT

Bixa orellana L. (family Bixaceae), commonly known as annatto or lipstick tree, is an economically and medicinally important plant native to tropical America and widely cultivated in tropical and subtropical regions. Traditionally, its leaves, seeds, bark, and roots have been used to treat various ailments, including gastrointestinal disorders, skin diseases, fever, urinary tract infections, jaundice and snakebite. Phytochemical studies have identified a diverse range of bioactive compounds, including alkaloids, flavonoids, terpenoids, saponins, glycosides, steroids, and tannins. The plant is particularly valued for its natural pigments, bixin and norbixin, which are concentrated in the seed and extensively used in the food, cosmetic, and pharmaceutical industries. Pharmacological investigations have demonstrated significant antibacterial, antifungal, anti-inflammatory, and antioxidant activities, supporting its traditional medicinal applications. This review summarizes the botanical characteristics, phytochemical composition and therapeutic potential of *B. orellana*, highlighting its importance as a promising natural resource for phytotherapeutic and pharmaceutical applications.

KEYWORDS: *Bixa orellana* L., pharmacognostic, medicinal uses.

INTRODUCTION

The *Bixa orellana* L. (family Bixaceae) is an evergreen shrub or small tree of economic importance, native to tropical America and widely grown in tropical and subtropical regions of the world (Lorenzi and Matos, 2002; Rivera-Madrid et al., 2016). The plant is known by common names annatto, lipstick tree and Sinduri and has been widely used in traditional medicine for its vast therapeutic properties (Kirtikar and Basu, 1999; The Wealth of India, 1988).

The species is best known for its bright red-orange seed arils containing the apocarotenoid pigments bixin and norbixin. These natural pigments are commercially valuable, safe and stable and are widely used as colorants in the food, cosmetic, pharmaceutical and textile industries (Mercadante et al., 1997; Rivera-Madrid et al., 2016).

Apart from industrial significance, different parts of the plant like leaves, seeds, bark, roots and flowers have been traditionally used for the treatment of fever, dysentery, diarrhoea, skin diseases, jaundice, urinary tract infections, kidney disorders, inflammation, headache, haemorrhage and snakebite in many tropical countries (Kirtikar and Basu, 1999).

Phytochemical studies have shown that B. orellana has a wide range of bioactive compounds, including carotenoids (bixin and norbixin), flavonoids, phenolic acids, terpenoids, tocotrienols, steroids, tannins, saponins, alkaloids and essential oils (Alves de Lima et al., 2003; Rivera-Madrid et al., 2016). These phytochemicals are responsible for the different biological activities of the plant like antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, hepatoprotective, wound healing and anticancer (Fleischer et al., 2003; Venugopalan et al., 2011; Rivera-Madrid et al., 2016).

Recent pharmacological studies have provided scientific evidence supporting many of the traditional medicinal uses of *B. orellana*, highlighting its potential as a valuable source of natural bioactive compounds for pharmaceutical and nutraceutical applications (Rivera-Madrid et al., 2016; Shahid-ul-Islam et al., 2016). Therefore, a comprehensive understanding of its botanical characteristics, phytochemical composition, ethnomedicinal significance, and pharmacological properties is essential for promoting its sustainable utilization and future therapeutic development.

History

Bixa orellana L., commonly known as annatto or the lipstick tree, has a long history of cultural, medicinal and commercial significance. The plant is believed to have originated from Brazil and is widely cultivated across tropical regions of the America, Africa and Asia, Philippines, Hawaiian Islands and tropical regions of Central and South America, where its seeds were traditionally used by indigenous communities as a natural body paint, cosmetic, insect repellent, and medicinal remedy. Following European exploration of the Americas during the sixteenth century, annatto spread to Asia, Africa, and other tropical regions, where it became an important economic crop because of its valuable natural pigments (Michelangeli 2010).

In India, the bright red pigment obtained from the seed aril has traditionally been associated with sindur (vermilion), the sacred red powder worn by married Hindu women as a symbol of marital status, prosperity, and longevity. The ritual of applying sindur along the hair parting and forehead has been practiced for centuries and is regarded as highly auspicious in Hindu culture. Natural sindur prepared from plant-derived pigments, including annatto, has attracted attention as a safer alternative to synthetic vermilion, which may contain harmful chemicals capable of causing scalp irritation and allergic reactions (Pathak, 2007).

Historically, annatto has also been valued as one of the oldest natural colorants used by humans. The pigments bixin and norbixin, extracted from the seed aril, have been extensively employed as natural coloring agents in the food industry, particularly for dairy products, confectionery, bakery products, processed foods, and beverages. Because of their safety and natural origin, these pigments are also widely used in pharmaceutical formulations, including ointments and plasters (Ulbricht et al., 2012).

Beyond the food and pharmaceutical industries, annatto dye has found extensive applications in cosmetics and other commercial products. It has traditionally been used in hair oils, soaps, nail polish, shoe polish, floor polish, furniture lacquer, brass lacquer, and decorative cosmetics, as well as for dyeing textiles, wool, leather, and calico fabrics (Ethnobotany Resource; Ulbricht et al., 2012). Although annatto is generally recognized as a safe natural colorant, rare cases of hypersensitivity and allergic reactions have been reported in susceptible individuals, warranting careful evaluation of its use in food and cosmetic products (Ulbricht et al., 2012).

The long history of *Bixa orellana* reflects its remarkable cultural, medicinal, and industrial importance, making it one of the world's most valuable natural dye-producing plants.

Botanical description

Bixa orellana L. is tropical plants that propagate in warm temperatures ranging from 32–38°C under dry climatic conditions. It grows well in a wide range of soil types, from loamy to lateritic soils. Propagation through budding and stem cuttings treated with indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) has been reported to be the most effective method for successful establishment. Phosphorus is considered an essential nutrient for optimum growth and cultivation of this plant (Nag et al., 2014). *Bixa orellana* is the most widely cultivated and economically important species (Yoke et al., 2013).

Bixa orellana is an evergreen shrub or small tree, usually 2–8 m in height, with a trunk reaching up to 10-15 cm in diameter. The bark is light to dark brown, tough, smooth and lenticellate. The inner region of bark is an orange-colored sap with a slightly bitter taste. Young twigs are green and covered with rusty reddish-brown scales, gradually becoming dark brown with maturity. The leaves are spirally arranged, simple, stipulate, and ovate, measuring 7.5–24 × 4–16 cm. They are shallowly cordate to truncate at the base, long-acuminate at the apex, dark green on the upper surface, and greyish or brownish beneath (Gordon et al., 2001; Wink, 2000; Kumaran K, 2014.; Fredrick et al., 2018).

The flowers are arranged in terminal branched panicles bearing 8–50 flowers and are covered with reddish-brown scales. Number of petals 4–7 are obovate, 2–3 × 1–2 cm in size, and vary in colour from white to pale pink. The fruit is a spherical to broadly ovoid capsule measuring 2–4 × 2–3.5 cm, flattened, two-valved, and densely covered with long soft bristles. At maturity, the capsules turn from green to greenish-brown or reddish in colour. Each capsule contains numerous obovoid to angular seeds approximately 4.5 mm long, enclosed by a bright orange-red fleshy aril rich in natural pigments (Panda et al., 2016).

The seeds measure approximately 0.3–0.5 cm in length and 0.2–0.3 cm in diameter, with shapes varying from pyramidal to nearly conical (Oliveira et al., 1996). The number of seeds per capsule generally ranges from 30 to 60, depending on the genotype and growing conditions (Alonso et al., 2004).

Microscopic examination of the leaves revealed an average stomatal number of 10.8 with a stomatal index of 9.34 on the lower epidermis, whereas the upper epidermis exhibited an average stomatal number of 14.0 and a stomatal index of 12.6, indicating a comparatively higher stomatal density on the upper surface. The average palisade ratio was recorded as 5.83

at the leaf base, 5.70 in the middle region, and 5.30 at the apex. The mean vein-islet number and veinlet termination number were 8.4 and 10.2, respectively which serve as important pharmacognostic parameters for the identification and standardization of *B. orellana* leaves (Panda et al., 2018; Kokate, 2008).

Origin and Vernacular Names

Bixa orellana L., commonly known as annatto or the lipstick tree, is native to the tropical regions of Central and South America, particularly Brazil, and is now widely cultivated and naturalized throughout tropical and subtropical regions of the world, including India, Malaysia, Indonesia, Peru, Mexico, Ecuador, Kenya, and several East African countries (Fredrick et al., 2018; Mata et. al. 2012). The species was named in honor of the Spanish explorer Francisco de Orellana, the first European to navigate the Amazon River, while the generic name Bixa is believed to have originated from a native South American vernacular term (Fredrick et al., 2018).

Owing to its extensive geographical distribution and long history of traditional use, *B. orellana* is known by numerous vernacular names across different countries and languages. The diversity of these names reflects its cultural, medicinal and economic importance worldwide.

Bixa orellana L. is widely distributed throughout tropical and subtropical regions of the world and is known by numerous vernacular names that reflect its geographical distribution and cultural importance. In North America, particularly in Spanish- and Portuguese-speaking regions, the plant is commonly known as Annatto and Achiote (Banajata.html; *Achiote Bixa orellana*). In Mexico, it is referred to as Changuarica**,** K'uzub**,** and Pumacua (Banajata.html). In Brazil, the plant is popularly known as Annatto or Urucu (Daniela de Araujo Vilar *et al.*), while in Germany it is recognized as Urucum (Bixa orellana database). In the French West Indies, it is known as Roucou, and in Colombia as Achiot (*Bixa orellana* database). In India, the common English names Annatto and Lipstick Tree are widely used (Fredrick *et al.*, 2018).

The plant is also known by a variety of regional names across different Indian states and languages. In Manipur, it is called Ureirom, whereas in Kannada it is known as Rangamali (Khare, 2007). In Tamil, the names Jabara, Manjitti**,** and Sappiravirai are commonly used (Plants Profile for *Bixa orellana*). The Malayalam name is Kurannumannal, the Marathi name is Sendri, the Gujarati name is Sinduri, the Assamese name is Jolandhar, and in Bengali it is known as Latkan (Plants Profile for *Bixa orellana*; Fredrick *et al*., 2018).

The wide diversity of vernacular names associated with *B. orellana* highlights its broad geographical distribution and long history of use by different communities. These names reflect the plant's cultural, ethnobotanical, and economic importance, particularly as a medicinal plant, a natural source of dye and food colorant, and an ornamental species. The extensive recognition of *B. orellana* across different regions further emphasizes its significance in traditional healthcare systems and its continued value in the food, pharmaceutical, and cosmetic industries.

Ethnomedicinal Uses

Medicinal plants have been used for the prevention and treatment of human diseases since ancient times and continue to play an essential role in primary healthcare worldwide (Aslam and Afridi, 2018). According to Willis (2017), approximately 28,187 plant species are recognized for their medicinal value, and nearly 50% of modern pharmaceutical drugs are derived directly or indirectly from natural products (Vilar et al., 2014).

Bixa orellana L. is one of the most important medicinal plants of the family Bixaceae, with a long history of traditional use in tropical and subtropical countries. Various parts of the plant, including the leaves, bark, roots, seeds, and stem, have been extensively utilized in traditional systems of medicine for the treatment of a wide range of diseases.

The leaves are commonly used as a decoction to treat vomiting, nausea, sore throat, urinary disorders, stomach ailments, headache, muscular pain, skin diseases, and diabetes. They are also reported to possess antibacterial, anti-inflammatory, antiseptic, antihaemorrhagic, hypocholesterolemic, diuretic, wound-healing, antimicrobial, hypotensive, digestive stimulant, and aphrodisiac properties. In several traditional practices, leaf extracts are also used to neutralize the effects of snake venom (Morrison et al., 1991; Chopra et al., 1956; Fleischer et al., 2003; Ulbricht et al., 2012; Giorgi et al., 2013).

The bark has traditionally been used as a febrifuge and antipyretic and for the treatment of gonorrhoea, headache, asthma, blood disorders, and excessive phlegm (Chopra et al., 2002; Prajapati et al., 2003; Khare, 2011; Hajoori et al., 2013).

The roots are used in traditional medicine to manage epilepsy, dysentery, fever, jaundice, diabetes, hypertension, liver disorders, sore throat, gonorrhoea, influenza, and urinary disorders because of their reported diuretic and therapeutic properties (Castello et al., 2002; Yoke et al., 2013; Alves et al., 2015).

The seeds are considered astringent and febrifugal and have been widely employed for the treatment of gonorrhoea, dysentery, epilepsy, kidney disorders, skin diseases, pharyngitis, bronchitis, and inflammatory conditions. Seed preparations are also used as mosquito repellents, haemostatic agents, diuretics, antipruritic agents, and possess reported hypoglycaemic, hypocholesterolaemic, analgesic, anti-inflammatory, antimicrobial, and antileishmanial activities (Khare, 2007; Giorgi et al., 2013; Vilar et al., 2014).

The stem has been traditionally used for the treatment of eye irritation and as a remedy against snakebite, particularly in parts of Malaysia, India, Brazil, and South America (Irobi et al., 1996; Fleischer et al., 2003; Nunez et al., 2004).

In addition to the medicinal uses of individual plant parts, the whole plant has been reported to exhibit diverse pharmacological activities, including anticlastogenic, antiemetic, antibilious, analgesic, anticonvulsant, antidiarrhoeal, antidiabetic, cardioprotective, diuretic, antipyretic, antiperiodic, antiulcer, and antiprotozoal properties. These activities support its extensive use in the management of gastrointestinal disorders, neurological diseases, diabetes mellitus, cardiovascular disorders, and infectious diseases (Villar et al., 1997; Barrio et al., 2004; Shilpi et al., 2006; Asokkumar Kuppusamy et al., 2012; Hajoori et al., 2013).

Furthermore, *B. orellana* hairy root cultures have demonstrated moderate antimalarial activity with low toxicity toward mammalian cells, suggesting their potential as a promising source of novel antimalarial compounds (Bo Zhai et al., 2014).

PHYTOCHEMICAL COMPOSITION

Preliminary Phytochemical Analysis

Preliminary phytochemical screening of *Bixa orellana* L. has revealed the presence of a diverse range of bioactive secondary metabolites, supporting its extensive use in traditional medicine. Qualitative analyses of seed extracts have demonstrated the presence of saponins, steroids, terpenoids, tannins, glycosides, carbohydrates, and flavonoids**,** whereas alkaloids and resins were either absent or detected in negligible amounts (Harborne, 1973; Harborne, 1998; Abayomi, 2014; Telrandhe et al., 2016; Fredrick et al., 2018).

Further phytochemical investigations of methanolic extracts have shown that both leaf and seed extracts contain steroids, flavonoids, tannins, saponins, and proteins, indicating that methanol is an efficient solvent for extracting biologically active constituents (Tamilselvi et al., 2011; Bhatnagar et al., 2015; Sangvikar et al., 2015; Gupta, 2016).

Comparative phytochemical studies on different plant parts have demonstrated variations in the distribution of secondary metabolites. Alkaloids have been reported in the leaves and roots but are generally absent in seeds. Flavonoids, tannins, triterpenoids, and carbohydrates are consistently detected in most plant parts, whereas proteins and saponins show organ-specific distribution. Fats and oils are predominantly present in the seeds owing to their high lipid content. These findings indicate that the phytochemical composition of *B. orellana* varies according to the plant part and extraction method employed.

Total Phenolic Content

Phenolic compounds are among the most important secondary metabolites synthesized by plants and play a crucial role in plant defense and human health (Huang et al., 2010). Structurally, phenolic compounds possess one or more hydroxyl groups attached to an aromatic ring and are widely distributed throughout plant tissues (Young, 2010). In recent years, these compounds have attracted considerable scientific interest because of their broad spectrum of biological activities and potential therapeutic applications (Han et al., 2007).

Plant phenolics exhibit diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, cardioprotective, and anticancer activities. Their antioxidant potential is primarily attributed to their ability to scavenge reactive oxygen species (ROS), donate hydrogen atoms or electrons, chelate transition metal ions, and inhibit lipid peroxidation (Young, 2010). Numerous in vitro studies have demonstrated that polyphenols effectively complement both enzymatic and non-enzymatic antioxidant defense systems, thereby protecting cells against oxidative stress-induced damage and reducing the risk of chronic diseases (Tsao, 2010).

Several studies have reported appreciable levels of total phenolic compounds in different parts of *B. orellana*, particularly in the leaves, suggesting that the plant is a valuable source of natural antioxidants. The phenolic content is influenced by factors such as plant part, geographical origin, extraction solvent, and extraction technique. The abundance of phenolic compounds contributes significantly to the antioxidant and therapeutic potential of *B. orellana*, supporting its traditional medicinal uses and highlighting its promise as a source of natural bioactive compounds for pharmaceutical and nutraceutical applications.

Fluorescence Analysis

Fluorescence analysis is a rapid, reliable, and widely accepted pharmacognostic technique used for the identification and quality assessment of powdered herbal materials. Many plant constituents exhibit characteristic fluorescence when exposed to ultraviolet (UV) radiation or after treatment with specific chemical reagents. The fluorescence behaviour of plant powders therefore serves as an important diagnostic parameter for detecting adulteration and ensuring the authenticity of herbal drugs (Ansari et al., 2006; Kulkarni et al., 2011).

Several studies have investigated the fluorescence characteristics of *B. orellana* leaf and seed powders using different chemical reagents under visible light and ultraviolet light (254 and 366 nm). The powdered leaves exhibited distinct colour changes ranging from green, greyish-yellow, orange-red, brown, golden-yellow, and dark green depending on the reagent and illumination conditions. Similarly, seed powder showed characteristic fluorescence responses, producing shades of brown, reddish-brown, yellowish-brown, pinkish-brown, dark green, and dark brown after treatment with various reagents. These characteristic fluorescence patterns provide valuable pharmacognostic markers for the identification and quality control of *B. orellana* (Sangvikar et al., 2015; Nanna et al., 2013; Birajdar et al., 2014; Panda et al., 2016).

Extractive Values

Extractive values are important physicochemical parameters used to evaluate the quality and purity of crude herbal drugs. They provide an estimate of the quantity of active constituents extracted by different solvents and indicate the nature of phytochemicals present in the plant material. The extractive value varies with solvent polarity and extraction technique, making it a useful indicator for selecting appropriate solvents for phytochemical and pharmacological investigations.

Different extraction methods have been employed for *Bixa orellana* L. Harborne (1998) described successive solvent extraction using petroleum ether, chloroform, ethyl acetate, and methanol to obtain phytochemical fractions of varying polarity. Panda et al. (2016) extracted *B. orellana* leaves using a Soxhlet apparatus with hexane, ethyl acetate, and methanol, followed by concentration of the methanolic extract under reduced pressure using a rotary evaporator. Similarly, Abayomi et al. (2014) and Telrandhe et al. (2016) determined the water-, alcohol-, petroleum ether-, and chloroform-soluble extractive values according to the procedures described in the Indian Pharmacopoeia. Among the seed extracts, the water-soluble extractive value (21.5% w/w) was the highest, followed by the alcohol-soluble extractive value (13.6% w/w), petroleum ether-soluble extractive value (9.7% w/w), and chloroform-soluble extractive value (7.5% w/w). The relatively high aqueous extractive value suggests the abundance of polar phytoconstituents in *B. orellana* seeds.

Ash Values

Ash values are important quality-control parameters used to determine the purity of crude plant materials and to detect contamination with inorganic matter such as soil, sand, and siliceous materials. Total ash, acid-insoluble ash, and water-soluble ash are routinely determined according to the methods prescribed in the Indian Pharmacopoeia.

Studies on *B. orellana* seeds have reported a total ash value of 6.55% (w/w), water-soluble ash of 2.69% (w/w), and acid-insoluble ash of 1.58% (w/w) (Panda et al., 2016; Telrandhe et al., 2016; Radhika et al., 2010). These values indicate relatively low levels of inorganic impurities and provide useful reference standards for the authentication and quality evaluation of *B. orellana*.

Moisture Content and Foreign Organic Matter

Moisture content is a critical parameter affecting the stability, shelf life, and microbial quality of herbal drugs. It is commonly determined by the loss-on-drying (thermogravimetric) method, in which the sample is dried at a constant temperature until a constant weight is achieved, following the procedures recommended by the Indian Pharmacopoeia (Indian Pharmacopoeia, 1985; Radhika et al., 2010; Fredrick et al., 2018; Telrandhe et al., 2016).

Foreign organic matter analysis is performed to assess the presence of extraneous materials such as stems, soil particles, insects, and other plant debris that may adversely affect the quality of crude drugs. For *B. orellana* seeds, the foreign organic matter and moisture content have been reported as 2.5% (w/w) and 15.82% (w/w), respectively (Telrandhe et al., 2016).

Antimicrobial activity

Several studies have demonstrated that *Bixa orellana* L. possesses significant antibacterial activity against a broad spectrum of Gram-positive and Gram-negative bacteria. The antibacterial efficacy varies depending on the plant part, extraction solvent, and test microorganism.

Tamil selvi et al. (2014) reported that leaf extracts exhibited greater antibacterial activity than seed extracts, while root extracts also showed higher activity than seed extracts. Among the tested bacterial pathogens, *Salmonella typhi* and *Acinetobacter* spp. were the most susceptible to the leaf extract. In the paper disc diffusion assay, the inhibition zones produced by the plant extracts ranged from 8.0 to 18.0 mm**,** whereas the positive control, streptomycin (10 µg/mL), produced inhibition zones ranging from 16.0 to 25.0 mm**.**

Similarly, Giridhar et al. (2012) evaluated the antibacterial activities of ethanolic, methanolic, acetone, and dimethyl sulfoxide (DMSO) extracts prepared from the leaves and fruit capsules of *B. orellana*. The extracts exhibited significant inhibitory activity against several clinically important bacterial pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*, as determined by the disc diffusion method.

The plant extracts have been reported to inhibit both Gram-positive and Gram-negative bacteria, with minimum inhibitory concentration (MIC) values as low as 15.62 µg/ml, indicating the presence of potent antibacterial constituents (Marjorie, 1999; Tamil selvi et al., 2014). Comparative studies have also shown that methanolic extracts generally produce higher extraction yields and stronger antibacterial activity than acetone extracts**,** suggesting that methanol is more effective in extracting bioactive antimicrobial compounds (Ongsakul et al., 2009; Shilpi et al., 2006).

The antibacterial activity of *B. orellana* is largely attributed to its rich phytochemical composition. Flavonoids are believed to exert their antimicrobial effects by disrupting bacterial cell membranes through interactions with membrane proteins and other cellular components, leading to increased membrane permeability and cell death (Marjorie, 1999). Saponins also contribute to antibacterial activity by increasing membrane permeability, resulting in leakage of intracellular proteins, enzymes, and other essential metabolites from bacterial cells (Zablotowicz et al., 1996). Usmani et al. (2022) evaluated the antibacterial activity of *Bixa orellana* 70% ethanolic leaf extract exhibited the highest antibacterial activity, producing a 19 mm zone of inhibition against S. aureus and a maximum inhibition zone of 40 mm against P. aeruginosa when combined with antibiotics at a 100 mg dose. The study concluded that the ethanolic extract possesses superior antibacterial potential compared to aqueous, ethyl acetate, and chloroform extracts, indicating that ethanol is the most effective solvent for extracting antibacterial phytoconstituents from *B. orellana* leaves.

Overall, the available evidence suggests that *B. orellana* is a promising natural source of antibacterial compounds with potential applications in the development of novel phytotherapeutic agents for the treatment of bacterial infections.

Antioxidant

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense system, plays a crucial role in the development of numerous chronic diseases. Consequently, natural antioxidants derived from medicinal plants have attracted considerable attention owing to their potential health benefits. Several studies have demonstrated that *Bixa orellana* L. possesses significant antioxidant activity, which is primarily attributed to its rich content of phenolic compounds, flavonoids, carotenoids, and other bioactive phytochemicals.

Yoke et al. (2013) reported that *B. orellana* seed extracts exhibited significant in vitro antioxidant activity using both 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing power assays, even at relatively low concentrations. Similarly, Abayomi et al. (2014) demonstrated that the free radical scavenging activity of annatto seed extract ranged from 5.5% to 48.9%**,** compared with 2.9% to 41.5% for ascorbic acid over concentrations of 0.25–2.5 μg/ml. The ferric reducing antioxidant power also exhibited a strong concentration-dependent relationship **(**R^2 = 0.9986), comparable to that of ascorbic acid **(**R^2 = 0.9934**)**, indicating that *B. orellana* seeds are a promising natural source of antioxidants (Abayomi et al., 2014; Giorgi et al., 2013).

The antioxidant capacity of *B. orellana* has been closely associated with its phenolic content. Extracts prepared using highly polar solvents generally exhibit greater free radical scavenging activity because of their higher concentrations of phenolic compounds. Cardarelli et al. (2008) and Chiste et al. (2011) reported total phenolic contents ranging from 0.30 to

1.84 mg gallic acid equivalents (GAE)/g dry seed, depending on the extraction solvent, and observed that extracts with higher phenolic contents displayed stronger antioxidant activity.

In addition to seed extracts, the leaves of *B. orellana* have also demonstrated remarkable antioxidant potential. Several in vitro and in vivo studies have shown that leaf extracts possess potent free radical scavenging activity, with a reported DPPH IC_{50} value of 22.36 µg/ml, indicating strong antioxidant efficacy (Shilpi et al., 2006; Antunes et al., 2005; Junior et al., 2005; Kovary et al., 2001; Martinez-Tomé et al., 2001 and Tonny et. al. 2024).

Among the various antioxidant assays, the ABTS and DPPH methods are the most widely employed for evaluating the antioxidant capacity of plant extracts. The ABTS assay is particularly useful for determining the total antioxidant capacity of both hydrophilic and lipophilic compounds, whereas the DPPH assay is highly sensitive for detecting radical-scavenging activity in hydrophilic fractions at low concentrations (Aarland et al., 2016; Raddatz et al., 2016).

Overall, the available evidence indicates that *B. orellana* is an excellent source of natural antioxidants. Its strong radical-scavenging and reducing activities largely attributed to phenolic compounds, flavonoids, and carotenoids, support its traditional medicinal use and highlight its potential application in the development of antioxidant-rich nutraceuticals and pharmaceutical products.

INDUSTRIAL APPLICATIONS

Bixa orellana L. is one of the most economically important natural dye-producing plants and ranks among the leading natural food colorants used worldwide (Morton and Ledin, 1952). The characteristic apocarotenoid pigments, bixin and norbixin, extracted from the seed aril, are extensively used as natural food-grade colorants because of their excellent colouring properties, low toxicity, and consumer preference for naturally derived additives (Ganju and Ganju, 2014). These pigments are widely incorporated into dairy products, confectionery, bakery products, processed foods, edible oils, margarine, butter, cheese, ice cream, and candies, owing to their lipid solubility and attractive yellow to orange-red colour (Satyanarayana et al., 2003; Hajoori et al., 2013; Venugopalan et al., 2011; Shahid-ul-Islam et al., 2016; Srineeraja, 2017).

Beyond the food industry, annatto pigments have gained considerable importance in the cosmetic, textile, leather, and pharmaceutical industries. The natural dye is commonly used in the manufacture of lipsticks, soaps, hair oils, nail polishes, pharmaceutical ointments, and the traditional Sindoor (vermilion) because of its natural origin, biocompatibility, and vibrant colour (Abayomi et al., 2014; Kapoor et al., 2008; Pandey, 2018). In the textile and leather industries, annatto serves as an eco-friendly dye for colouring cotton, silk, wool, leather, and other natural fibres. Furthermore, recent studies have explored its application in advanced materials, including dye-sensitized solar cells and other sustainable industrial products, thereby expanding its commercial significance (Gupta, 2016; Shahid-ul-Islam et al., 2016).

The pharmaceutical industry also utilizes annatto pigments as natural colouring agents in ointments, capsules, tablets, and topical formulations because of their safety and antioxidant properties. The growing demand for plant-based ingredients has further increased the industrial relevance of *B. orellana*.

In addition to pigment production, the by-products of annatto processing have potential industrial value. The wood of *B. orellana* is lightweight (specific gravity approximately 0.4), soft, and of limited commercial timber value; however, it was traditionally used as a fire-starting material because of its ease of ignition. Moreover, the fruit pericarp, which remains after pigment extraction, has been identified as a promising renewable biomass resource and may be utilized as a potential biofuel, contributing to waste valorization and sustainable utilization of annatto processing residues (Parimalan et al., 2007).

Overall, the extensive applications of *B. orellana* in the food, cosmetic, textile, pharmaceutical, leather, and bioenergy sectors underscore its economic importance and highlight its potential as a sustainable natural resource for diverse industrial applications.

CONCLUSION

Bixa orellana L. is an important medicinal and industrial plant, with a long tradition of use and high economic value. The plant is rich in a variety of bioactive phytochemicals such as carotenoids (bixin and norbixin), flavonoids, phenolic compounds, terpenoids, tannins, saponins and alkaloids responsible for its diverse pharmacological activities. Scientific studies have confirmed the presence of antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, hepatoprotective, wound healing and other therapeutic properties in different parts of the plant, thus authenticating its traditional medicinal uses.

B. orellana is not only used for its medicinal value but also as a natural colorant in the food, cosmetic, pharmaceutical, textile, and leather industries due to its safety, biodegradability and excellent colouring properties. The commercial importance of this product has also been boosted by the rising demand for natural products.

Despite this considerable progress in phytochemistry and biological activities of *B. orellana*, more studies should be conducted to explore other parts of the plant, isolate the active constituents, elucidate their mechanisms of action and assess their clinical efficacy and safety. To summarize, the available evidence suggests that *B. orellana* is a promising source of natural bioactive compounds with a high potential for the development of new phytopharmaceuticals, nutraceuticals and environmentally friendly industrial products.

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