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BIXAORELLANA: PHYTOCHEMISTRY, EXTRACTION TECHNIQUES AND PHARMACOLOGICAL ACTIVITIES - A REVIEW

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ABSTRACT

Bixa orellana L. (family Bixaceae), commonly known as annatto or achiote, is a widely distributed medicinal plant native to the Americas and cultivated across tropical regions including India, Mexico, Brazil, and East Africa. This review comprehensively examines the phytochemical composition, extraction methodologies, and pharmacological activities of *B. orellana*. The plant is renowned for its carotenoid pigments—bixin (fat-soluble) and norbixin (water-soluble)—which constitute the commercially valuable annatto dye, accounting for approximately 70% of natural dyes consumed globally. Beyond its industrial applications, *B. orellana* possesses a rich phytochemical profile comprising terpenoids (including geranylgeraniol), flavonoids, alkaloids, tannins, saponins, sterols, and essential oils distributed across various plant parts (seeds, leaves, roots, and flowers). Extraction techniques range from conventional solvent-based methods (using ethanol, methanol, ethyl acetate, chloroform, and hexane) to advanced approaches such as supercritical CO_2 extraction, pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), and ultrasound-assisted techniques, each yielding varying quantities of bixin and norbixin depending on solvent polarity and temperature conditions. Pharmacological investigations have demonstrated extensive therapeutic potential, including antimicrobial activity against Gram-positive and Gram-negative bacteria, antifungal effects particularly against *Candida* species, antioxidant properties evidenced by DPPH and ABTS scavenging assays, anticancer activity against various cell lines (B16F-10 melanoma, HeLa, A549 lung cancer, MCF-7 breast cancer), antidiabetic effects through α-glucosidase and α-amylase inhibition, anti-inflammatory, analgesic, anticonvulsant, antileishmanial, and wound-healing activities. Recent developments include green synthesis of silver nanoparticles from plant extracts, enhancing bioactivity. Despite substantial preclinical evidence, clinical translation remains limited. This review underscores the need for standardized extraction protocols, rigorous clinical trials, and nanoparticle-based formulations to fully harness the therapeutic potential of *B. orellana* as a safe, natural alternative in modern medicine.

KEYWORDS: *Bixa orellana*, Annatto, Phytochemistry, Bixin, Norbixin, Extraction techniques, Pharmacological activities, Antimicrobial, Antioxidant, Anticancer, Antidiabetic.

INTRODUCTION

Natural plants are safer and more effective medicinal use as compared to commercially available synthetic and toxic agents to treat different ailments.[1] According to the WHO, around 20000 plants species are used for medicinal purposes.[2]

Bixa orellana L., commonly known as Annatto, belongs to the family Bixaceae and genus Bixa. It is widely distributed across the globe. Native to the Americas area, grows well in lowlands and mountainous regions or higher elevation.[2]

Due to its wide distribution, *Bixa orellana* has different common names.[2] "Annatto" or "Achiote" in North America, "Changuarica", "Pumacua" in Mexico, "Urucu" in Brazil, "Latkan" in Hindi, Bengali, Oriya, "Rangamali" in Kannada, "Sinduri" in Gujarati, "Usrang" in Arabic.[2] The plant is also cultivated in Peru, Mexico, Ecuador, Indonesia, India, Kenya, East Africa and other hot regions.[3]

The height of *Bixa orellana* ranges from 3 to 5 meters; occasionally reach 10 meters. The short trunk 20-30 cm in diameter, has dark grey bark with vertical rows of

lenticels. The leaves, 10-20 cm long and 5-10cm wide, are alternate, pointed, green on both surfaces and have long petioles.[4] The plant has showy pink or white flowers, nearly 5 cm across. The flowers contain five petals and a dense mass of stamens in the centre. The fruits are red, spiny, 2-5 cm long and 2-valved and ovoid in shape.[2] The plant requires warm climate around 35^{o}C, dry climate and cannot tolerate wet and foggy climate.[5]

Its outer seed extract is utilized as a natural food colouring agent due to the pigments bixin and norbixin and yield a dye named "Annatto". Bixin is fat soluble and norbixin is water soluble. Annatto yields both water- and fat-soluble colorants depending on the extraction method, solvent and temperature due to which annatto or achiote is a highly valuable plant source for natural colorants. It is estimated that 70% of all the natural dyes consumed are derived from *B. orellana* dye. Annatto dye is also used to colour hair oils, shoe polishes, furniture, textile, soap, cosmetic, floor polishes and pharmaceutical ointments.[6]

Historically, the seed pulp was used as therapeutic to treat several ailments like constipation, burns, gonorrhoea and bleeding. The leaves, bark and root extract was used as an antidote for poisoning, particularly for snakebite in the Columbian region, known as antivenom agent.[7] The Brazilian utilized it as a moderate diuretic and for the treatment of heartburn.[8] The plant was also used to treat fever, asthma, jaundice, joint pain, sore throat, wound healing, etc in many other countries.[9]

Brazil is the largest producer of *Bixa orellana* seeds with the estimation of 15,000 metric tonnes in which half is used for domestic consumption and rest is for export. India produces about 500 metric tonnes.[10]

Bixa orellana plant has pharmacological and therapeutic properties which makes it a valuable and natural resource in traditional and modern medicine.[11] It shows antimicrobial, antibacterial, antifungal and antileishmanial properties against infections.[12,13] It also possesses anticonvulsant, analgesic, antinociceptive and spasmolytic activities, contributing to its effectiveness in pain management and neurological conditions.[14] The plant also cures metabolic diseases through its antidiabetic, anti-obesity effects, while its antioxidant and anti-inflammatory properties help to manage conditions such as colitis and bone loss.[15,16]

The plant has applications in treating gastrointestinal disorders as well as respiratory and cardiovascular conditions. It is also used in burn, wound healing, hair growth, weight loss, improving sleep and enhancing vitamin A activity. These vast medicinal benefits make annatto a promising plant for pharmacological research and therapeutic applications.[3]

PLANT PROFILE

Taxonomical classification

1. Kingdom: Plantae
2. Class: Magnoliopsida
3. Order: Malvales
4. Family: Bixaceae
5. Genus: Bixa
6. Species: *Bixa orellana*

Morphological character

1. Leaves: Spirally, simple, blade ovate
2. Margin: Entire
3. Odour: Characteristics
4. Taste: Slightly bitter taste
5. Apex and Base: Acute
6. Flower: Bisexual, regular, fragrant
7. Fruit: Globose or broadly to elongated ovoid capsule
8. Seed: Bright orange-red fleshy seed coat.[17,18]

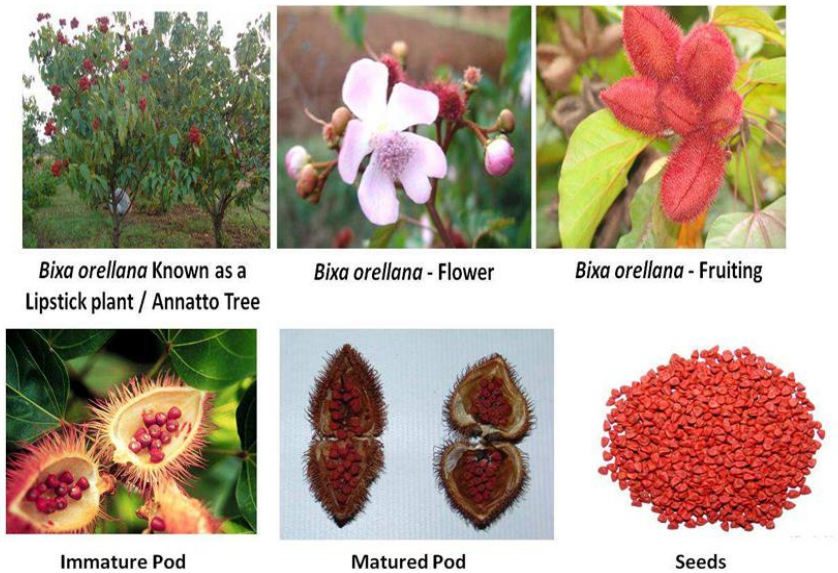

Fig. 1: *Bixa orellana* tree, flower, fruits, pods and seeds.

CHEMICAL PROFILE

Bixin was isolated in 1961 from the seeds of *Bixa orellana*. Its structure and stereochemistry were determined by NMR spectroscopy. Bixin belongs to the class of natural apocarotenoids, whose formation occurs by oxidative degradation of C40 carotenoids.[19] It is a diapocarotenoid that has two carboxylic group when dissolves in fat one of which is esterified.[20] The ester group is hydrolysed to produce norbixin, which is water soluble.

Carotenoids bixin and norbixin have cis and trans isomeric configurations. Under normal conditions cis forms of both carotenoids are unstable. It can be converted into trans configurations by heating the solution of cis bixin or norbixin, which is more stable and known as iso-bixin and iso-norbixin.[21] Bixin is a 25-carbon molecule with the chemical formula $C_{25}H_{30}O_4$ (M.W. = 394.51).[22]

Long-term trends are a highly unsaturated chemical with chromophores in its conjugated double bonds. Norbixin acts as a sodium or potassium salt in alkali and has a significant tinctorial value in water-based formulations. In practice, bixin and norbixin show better light stability than many other carotenoids, but they are both unstable in the presence of oxygen, just like other antioxidant carotenoids. Relative to other carotenoids, bixin and norbixin have good heat stability during food processing.[23]

In addition, the seeds also contain cellulose, sugars, essential oils, fixed oils, pigments, proteins, alpha and beta-carotene as well as tannins and saponins.[24] Other carotenoids present in minor proportions are β-carotene, cryptoxanthin, lutein, zeaxanthin, methylbixin, six apocarotenoids (C30 and C32), eight diapocarotenoids (C19, C22, C24, and C25), and a carotenoid derivative (C14). Geranylgeraniol, tocotrienol and eicosatrienoic acid, were also identified. Geranylgeraniol, a key oily ingredient of annatto seeds, accounts for 1% of dry seeds.

Bixa orellana has been described as the vegetable source with the highest concentration of terpenes like E-geranylgeraniol, which can account for up to 57 percent of total terpenes in the dry seed. It also contains lipids (linoleic acid, -linolenic and oleic acids in smaller proportions), amino acids (glutamate, aspartate, leucine), and phosphorus, iron, and zinc.[24-26]

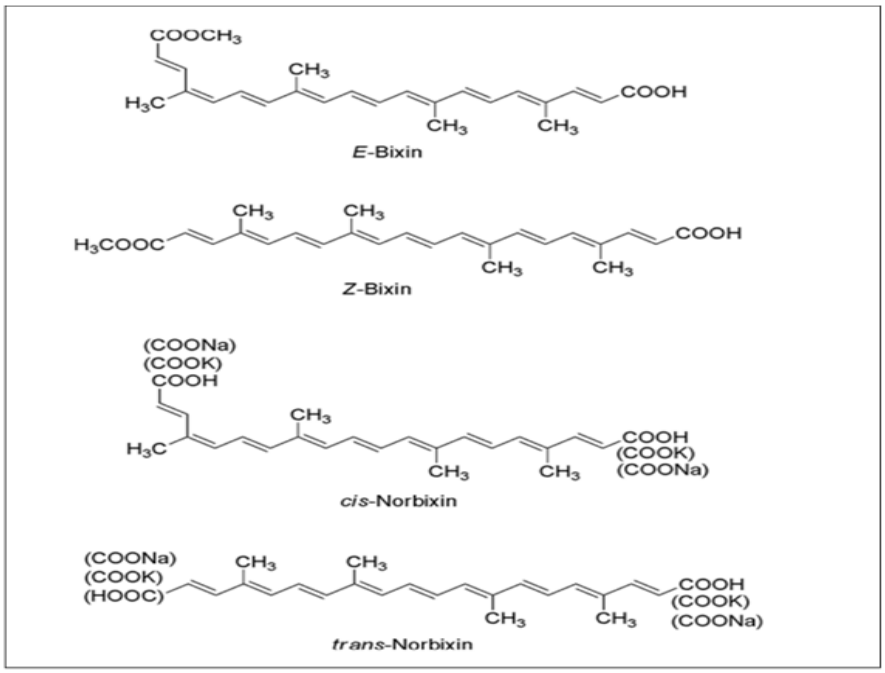

Fig. 2: Chemical structure of bixin and norbixin.

EXTRACTION

The extraction of annatto contains the factors such as the method of extraction, type of solvent and its quantity used, extraction time and temperature required selectivity of the certain component, amount of the sample used, efficiency of extraction and the nature and condition of the place. The nature of solvents is the most crucial to dictate the target compound's solubility and extraction efficiency. Depending on the kind of extraction, as well as the solvent and temperature utilized, water-soluble colorants (norbixin) and oil-soluble colorants (bixin) can be obtained.[27]

The commercial extraction of pigment from dried annatto seeds is primarily achieved through three methods: oil extraction, organic solvent extraction, and aqueous alkali extraction. Oil and organic solvent methods yield bixin as the primary colorant, whereas

aqueous alkali extraction saponifies the bixin to produce norbixin, a colorant frequently used in the dairy industry. Solvent extraction—utilizing polar solvents such as ethyl acetate, ethanol, or chloroform—is considered highly efficient and can yield microcrystalline bixin concentrates with 80–97% purity.[20,21,28]

Various scientific groups have studied various solvent systems and their combinations to improve extraction efficiency. The efficiency of the extraction process is heavily influenced by the complex microstructure of the vegetal matrix because it is made up of cells, intracellular and capillary gaps, and pores, the molecular structure, size, and shape have an impact on the extraction and can be improved through mechanical processes like grinding, as well as physical-chemical treatments.[29,30] Bixin content can range from 1.0 to 6.0 percent, depending on the cultivar and the environment in which it is cultivated; from 1 and 4%, depending on the quality of the seeds.[31,32]

Studies of Annatto pigment extraction have been carried out using supercritical CO_2 and CO_2 modified with several mixed chemicals (methanol, chloroform, and acetonitrile). It was shown that the entertainers or mixed chemicals increased the efficiency of extraction. Two extraction methods are known as Pressurized Liquid Extraction (PLE) and Low-Pressure Solvent Extraction (LPSE) in terms of temperature, pressure, solvent to feed ratio, with and without sonication, and solvent type was investigated.[33-35]

In other cases, advances in extraction with the help of Microwave have led to the development of techniques such as microwave hydrodistillation (MWHD), vacuum microwave hydrodistillation (VMHD), microwave-assisted hydraulic disturbance (MHG), microwave-assisted extraction (MAE), and solvent-free microwave extraction (SFME).[36,37]

Depending on the extraction process and temperature utilized, annatto extracts may contain varying quantities of bixin and norbixin. High-pressure liquid chromatography (HPLC), UV-VIS spectrophotometry (UV-VIS), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry can all be used to identify and quantify bixin and norbixin.[38,39]

PHYTOCHEMISTRY OF *BIXA ORELLANA*

The chemical components of *B. orellana* can vary according to the part of the plant, location area of the species or even according to edaphic, climatic and environmental factors, in general.[40] Considering all the plant organs of *B. orellana*, the main compounds found are the pigments of the carotenoid class, which includes bixin, as well as some terpenoids, tocotrienols and flavonoids.[41]

B. orellana has undergone phytochemical screening which has resulted in the several chemical compounds with various structural characteristics. Carotenoids, apocarotenoids, sterols, aliphatic compounds, monoterpenes, sesquiterpenes, triterpenoids and other chemical elements have all been found and isolated, primarily from the seeds, seed coat and leaves of *B. orellana*.[17]

Various solvents are used to determine different chemical compounds. The phytochemical screening of the crude aqueous extract of *B. orellana* indicates the presence of flavonoids, tannins, anthraquinones, saponins and terpenoids.

Acetone extract indicates the presence of terpenoids and glycosides. Methanol extract indicates the presence of tannins and glycosides. Ethanolic extract indicates the presence of tannins, flavonoids, saponins, steroids and terpenoids.

Hexane extract indicates the presence of glycosides. Terpenoids can be isolated in ether extract. Ethyl acetate extract indicates the presence of tannins, flavonoids, saponins, steroids and terpenoids. The Hydroethanolic extract of *B. orellana* (leaves) showed the presence of terpenes, flavonoids, tannins, coumarins and saponins and absence of alkaloids and anthraquinones.[42-45]

Table 1: Phytochemicals present in the type of solvents extract.

S. NO.	SOLVENTS	SEEDS	LEAVES
1	Aqueous extract	Flavonoids, Steroids, Cardiac Glycosides, Terpenoids and Anthraquinone	Alkaloids, Flavonoids, Cardiac glycosides, Steroids, Tannins and Terpenoids
2	Ethanolic extract	Alkaloids, Terpenoids, Flavonoids, and Anthraquinone	Flavonoids, Steroids, Cardiac Glycosides, Terpenoids, alkaloids, tannins, Triterpenoids, Sterols, Flavones,
3	Methanolic extract	Flavonoids, Terpenes, Saponins	Flavonoids, Cardiac Glycosides, alkaloids, tannins, Triterpenoids, Steroids, Sterols, Saponins, Flavones, Flavonoids
4	Chloroform extract	Glycosides, Anthraquinone glycosides, Proteins, Tannins, Steroids, Flavonoids, Saponins	Alkaloids, tannins, Triterpenoids, Steroids, sterols, saponins, Flavonoids, flavones
5	Ethyl acetate extract	Glycosides, Anthraquinone	Alkaloids, tannins, Triterpenoids, Steroids,

		glycosides, Proteins, Tannins, Triterpenoids, Steroids, Saponins, Flavonoids	Sterols, Saponins, Flavones, Flavonoids
6	Acetic acid extract	No phytocemicals	Alkaloids and Cardiac Glycosides
7	Petroleum ether extract	Tannins, Steroids, Alkaloids	No phytochemicals

Table 2: Chemical constituents present in different parts of plants.

S. NO.	Plant parts	Chemical constituents
1	Whole plant	Carotenoid bixin(80%), norbixin, bixaghanene, bixein, bixol, crocetin, ellagic acid, ishwarane, isobixin, phenylalanine, salicylic acid, threonine, tomentosic acid, and tryptophan
2	Leaves	Maslinic acid, gallic acid, pyrogallol, isoscutelarein, flavonoids, heterosides, sulphated derivates, diterpenes, gallic acid, pyrogallol & essential oil
3	Root	Triterpene, tomentosic acid
4	Seed pulp	73 essential oils were identified from seeds, ishwarane and geranylgeraniol as major components in 18.6 and 9.1 %. Carotenoid like Bixin, 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, Apo-ψ carotene, 9′Z-6′-ol Beta carotene, Bixin (red dye), Z-Carotene, Cryptoxanthin, Phytoene, Phytofluene, Geranylgeraniol, Lutein lycopenoate, Neurosporene, Norbixin, trans-bixin, Zeaxanthin, orellin (yellow dye) some terpenoids, tocotrienols, arenes and flavonoids including luteolin and apigenin (Z,E) farnesyl acetate, occidentalol acetate and spathulenol

PHARMACOLOGICAL ACTIVITIES

Bixa orellana is a plant with extensive traditional medicinal use that has been increasingly investigated for modern pharmacological applications. Multiple plant parts (leaves, seeds, roots, bark, flowers) show diverse biological activities across in vitro and animal models. It contains carotenoids, flavonoids, alkaloids and other phytochemicals that underlie antioxidant, metabolic, neuropharmacological, analgesic, anti-inflammatory, antimicrobial, antiparasitic and anticancer effects in preclinical studies. Leaves and seeds are most studied, with evidence for CNS depression, pain relief, GI protection, and broad antimicrobial activity.[17]

1. Antimicrobial activity

Valarezo et. al. extracted the essential oil from the *Bixa orellana* leaves by hydro distillation method with the yield of 1.3 ± 0.1 ml/kg. The characterization of essential oil was carried out by Gas chromatography coupled to mass spectrometry (GC-MS).A total of 56 chemical compounds were identified in the EO of leaves from *B. orellana*, which represent 99.25% of the total compositions. In this essential oil, strong to moderate Antibacterial activity was determined using the broth microdilution method, for which he used three Gram-positive cocci bacteria, a Gram-positive bacilli bacterium and three Gram-negative bacilli bacteria. The anticholinesterase (anti-AChE) activity was determined using the spectrophotometric method.[46]

Selvi A. et. al. extracted the leaf and sead of *Bixa orellana* from methanol by soxhlet extraction method. Methanol extract indicated the presence of steroids, flavonoids, tannins, saponins and proteins in both leaf & seed extracts of *B. orellana*. Extraction showed the in vitro antimicrobial activity. Leaf extract of *B. orellana* showed significant inhibition against all the tested bacteria and fungus, with highest inhibition zone (18±0.3 mm) against S. typhi, Acinetobacter sp., T. mentagrophytes and T. rubrum. Seed extract of *B. orellana* was comparitively less efficacious in most of the tested pathogens, except Brucella sp. which was appreciably inhibited (15±0.1 mm). Scanning Electron Microscopic (SEM) technique was treated with P. aeruginosa cells which revealed the disintegration & aggregation of cells after treatment with the leaf extract.[47]

Khambhaty et. al. synthesized the silver nanoparticles (AgNPs) by using *Bixa orellana* root extract. The nanoparticle was characterized by UV, SEM and XRD techniques in which UV showed surface plasmon resonance at 425 nm, SEM provided the size of AgNPs, 35-53 nm and X-ray diffraction spectrum exhibited 2θ values corresponding to the silver nanocrystal. FTIR analysis confirmed the presence of various amine, alcohol, and carboxylic acid groups. The root extract AgNPs exhibited superior antibacterial and antifungal activity as compared to the root extract only and was assessed against both Gram-positive and Gram--negative bacteria.[48]

Barik et. al. synthesized the silver nanoparticles (AgNPs) from the aqueous seed extract of *Bixa orellana*. AgNP was studied using techniques such as UV–visible spectroscopy, dynamic light scattering (DLS), Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and scanning electron microscopy (SEM). UV–vis confirmed formation of Ag nanoparticle where the average grain size was 54 nm as per SEM studies. The BOAgNPs was used to treat antibacterial activity against two Gram positive (Bacillus cereus,

Bacillus subtilis) and two Gram-negative (Escherichia coli, Shigella flexneri) bacteria. It also showed anti-cancerous and antioxidant activity.[49]

2. Anticancer activity

Kumar et. al. extracted the seed of *Bixa orellana* by petroleum ether. The presence of a bioactive component of PE extract was analyzed by GC-MS method. GC-MS analysis shows the presence of three anticancer compound geranylgeraniol, squalene and beta-sitosterol. The IC50 values for the B16F-10 melanoma cell were 145.98±7.49 µg/ml and 121.60±6.20µg/ml for 24h and 48h respectively. The study established that the seed extract by PE possessed potent anticancer activity and apoptotic inductive potential.[50]

Kusmita et. al. extracted the *Bixa orellana* seed and Isolated bixin from column chromatography has been confirmed using LC-MS/MS. Bixin and crude extract were examined on human lung cancer (A549), cervical cancer (HeLa), and breast cancer (MCF-7). Anti-proliferative activity revealed to be promising on both, the isolated pigment and crude extract.[51]

Valencia et. al. extracted the leaves and seed of *Bixa orellana* by methanol using soxhlet method. The antioxidant activity observed in the extracts of the seeds and leaves of *B. orellana* due to the presence of some phytochemicals, mainly flavonoids. The extracts of the seeds and leaves showed antiproliferative activity on the HeLa cell line.[52]

3. Antioxidant activity

Amorim et. al. extracted the leaves of *Bixa orellana* using aqueous, saline and hydroethanol. The analysis of phytochemicals was performed by TLC and HPLC. Ellagic acid and flavonoids were present in all extracts, which exerted antioxidant activity, with IC_{50} values ranging from 6.90 to 23.22 µg/mL for DPPH and $ABTS^{+}$. AE, HE, and SE inhibited the growth of *L. amazonensis* promastigotes, resulting in antileishmanial activity.[53]

Akthar et. al. extracted the flowers of *Bixa orellana* and characterized the extraction by performing HPTLC and LC-MS. LC-MS profiling revealed quercetin, kaempferol, genestin, etc. as key components of the flower extract and HPTLC profiling showed several major and minor metabolites at different Rf values. These compounds showed a significant results against oxidative stress using DPPH method.[54]

Valarezo et. al. extracted the essential oil from the *Bixa orellana* leaves by hydro distillation method with the yield of 1.3 ± 0.1 ml/kg. The characterization of essential oil was carried out by Gas chromatography coupled to mass spectrometry (GC-MS). . The strong antioxidant activity was found by ABTS acid radical cation and moderate in DPPH free radical scavenging capacity.[46]

4. Antifungal activity

Rodrigues et. al. extracted bixin from seeds of *Bixa orellana* by soxhlet method using hexane and chloroform and recrystallised by acetone. The characterization of bixin was done by TG/DTA, FTIR and ^{1}H NMR and confirmed predominance of the 9'-cis isomer. Quantitative analysis of the recrystallized product was carried out using an HPLC method. The purity of the bixin sample, purified and crystallized, was determined to be 95.86%. The compound exhibited moderate to significant antifungal activity against various Candida species, particularly C. glabrata and C. tropicalis. Bixin also inhibited virulence factors in C. albicans and cytotoxicity on Vero cells for C. glabrata and C. tropicalis.[55]

5. Antidiabetic activity

Pooja et. al. extracted the *Bixa orellana* leaves by ethanol using soxhlet apparatus. The IC_{50} values were found to be 398.51 µg/mL for sucrose and 78.41 µg/mL for maltase, indicating a dose-dependent inhibitory effect, resulting in α-glucosidase inhibitory potential. The inhibitory activity of EEBO against α-amylase came out good with an IC_{50} value of 133.48 µg/mL. This investigation suggested that the ethanol extract of *Bixa orellana* possessed significant anti-diabetic properties.[56]

Tonny et. al. extracted the *Bixa orellana* seed from methanol by soxhlet method. It was found that the seed extracts used at 500 mg/kg and 1000 mg/kg evidently reduced the blood glucose level as opposed to the diabetic control group, more importantly with the rats treated with standard, Metformin Hydrochloride. It also showed antioxidant activity by DPPH method.[57]

6. Other pharmacological activities

Muddapur et. al. extracted the leaves of *Bixa orellana* by ethyl acetate using soxhlet method. The extracted compound of bixa, revealing the presence of alkaloids, flavonoids, and phenols, found in satisfactory quantities during the quantitative analysis. The characterization of compound was done by TLC, column chromatography, and component analysis by GC–MS, which reported 18 components, and UV spectroscopy. A number of therapeutic applications for the extract were observed, including antibacterial activity, antidiabetic activity, antifungal activity, anti-lypase activity, anti-amylase activity, antioxidant activity and some other preliminary therapeutic activity.[58]

Yong et. al. extracted the leaves of *Bixa orellana* in aqueous medium. The extracted compound was characterized by GC-MS and several organic compounds were detected, having the major constituent of acetic acid. The extract exhibited the ability to suppress the inflammatory activities, and showed significant inhibition of histamine-induced paw edema, with maximal percentage of inhibition (60.25%).[59]

CONCLUSION

Bixa orellana plant serves as a rich biological reservoir of valuable phytochemicals. It is most famous for its high concentration of the carotenoid pigments bixin and norbixin which yield the highly valuable Annatto dye. Its seeds, leaves, roots, and flowers contain a complex matrix of terpenoids (such as geranylgeraniol), flavonoids, essential oils, and alkaloids.

Extensive preclinical research utilizing various extraction methods and solvent systems have confirmed that these phytochemicals drive a powerful and diverse pharmacological activity such as antimicrobial, anticancer, antioxidant, antifungal, antidiabetic activities.

Given the vast medicinal benefits and efficacy demonstrated across numerous in vitro and animal (preclinical) models, a primary focus is to advance this pharmacological research into clinical trials to develop safe, natural therapeutic applications for human medicine. Further research can also be done on increasing the effectiveness of the extract, by synthesizing nanoparticles of the same.

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