



## Pharmacognostical and Phytochemical Investigation of leaves of *Bixa orellana* Linn

Swati N.Deshmukh<sup>1\*</sup>, B.Shrivastava<sup>2</sup>, Pankaj Sharma<sup>2</sup>, Hemant Kumar Jain<sup>3</sup>, N.Ganesh<sup>4</sup>

<sup>1</sup>Department of Pharmacy, Alard college of Pharmacy, Marunje, Pune, (M.S), India.

<sup>2</sup>School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India.

<sup>3</sup>Sinhgad College of Pharmacy, Vadgaon, Pune, (MS), India.

<sup>4</sup>Jawaharlal Nehru Cancer Hospital and Research Centre, Idgah Hills, Bhopal, M.P., India.

\*Corresponding author's E-mail: [swatindeshmukh@rediffmail.com](mailto:swatindeshmukh@rediffmail.com)

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### ABSTRACT

*Bixa orellana* L. commonly known as annatto belonging to the family Bixaceae is valued for its food and medicinal uses. The literature survey reveals that in traditional systems of medicine, different parts (leaves, seeds) of *Bixa orellana*, a small tree seen throughout India, have been recommended for many more presumed beneficial uses. The present study dealt with the pharmacognostic and phytochemical investigation of *Bixa orellana* leaves. The leaves of *Bixa orellana* selected for the study is collected from Marunje Village of Pune District, MS, India. To mark its official standards it is necessary to study the plant in crude and powdered form which help to identify the different species of *Bixa orellana*. Pharmacognostical study of leaf shows the glossy cordate acuminate leaves are ever green with reddish veins with a thin long petiole. Leaves spirally arranged, simple, stipulate, ovate, shallow cordate to truncate at base, longly acuminate at apex, green or dark green above grey or brownish green beneath. Microscopically the leaf is dorsiventral, the midrib and the laminar region showed single layered epidermis on both the surfaces and covered with thick cuticle. The microscopy shows the presence of vascular bundle, collenchymas, spongy parenchyma and palisade cells. Physicochemical evaluation and fluorescence study was carried out and different extracts were investigated. For the presence of preliminary phytochemicals like, alkaloids, tannins, triterpenoids, steroids, sterols, saponins, flavones, flavonoids. The present study might be useful to provide the information in regards to the identification parameters and can possibly help to differentiate the drug from its other species/varieties.

**Keywords:** *Bixa orellana*, Fluorescence analysis, Macroscopy, Microscopy, Phytochemical study.

### INTRODUCTION

*Bixa orellana* L. commonly known as annatto, Bengali (latkan); English (lipstick tree, arnato tree, annatto tree; Hindi (latkan), Tamil (japhara) belonging to the family Bixaceae is valued for its food and medicinal uses. In developing countries people of native communities use this plant in folk medicine for the treatment of common infections in the form of decoctions, teas, juices etc.<sup>1</sup> *Bixa orellana* is an evergreen shrub or small tree, 2-8 m high; trunk up to 10 cm in diameter; bark light to dark brown, tough, smooth, sometimes fissured, lenticellate; inner bark pinkish towards the outside with orange sap, slightly bitter; twigs green with minute, rusty, reddish-brown scales, becoming dark brown. 'Bixa' is derived from a local South American name. The flowers are pollinated by honeybees, and the fruit matures 5-6 months later. Seed-grown plants take longer to flower than vegetatively propagated ones, and do so sparingly. Under favourable conditions, fruiting commences 18 months from planting or earlier, and full crops of seeds are obtained after 3-4 years. In Puerto Rico and the Virgin Islands, flowering occurs mainly in spring and fruiting chiefly in the summer.<sup>2</sup> The plant grows equally well in low lands and mountainous regions or areas of higher elevations.<sup>3</sup> It is native to the tropical America and is found in large quantities from Mexico to Ecuador, Brazil and Bolivia. This plant is cultivated in warm regions of the world, such as India, Sri Lanka and

Java mainly for the dye obtained from the seeds.<sup>4</sup> In India, the plant is cultivated and found wild especially in Western parts of the country. *Bixa orellana* (Bixaceae) is a tree reaching 4 to 6 meters. Leaves are entire, ovate, 8 to 20 cm long, 5 to 12 cm wide, with a broad and heart-shaped base, and a pointed tip. Its glossy cordate acuminate leaves are ever green with reddish veins with a thin long petiole. The young twigs are covered with rust colored scales and became bare when older. The flowers are white or pinkish, 4 to 6 cm diameter, 4 to 6 cm in diameter on terminal panicles. Capsules are ovoid or rounded, reddish brown, about 4 cm long and covered with long, slender and soft spines containing many small seeds covered with a dye-yielding red pulp.<sup>5-7</sup> Fruit is a capsule, reddish brown, soft and with bristly hairs. The two valved round fruits are approximately 4cm wide; appear in a variety of colors like scarlet, yellow, brownish yellow and bright red. When ripe, the fruits split open and reveal a numerous amount of small, fleshy seeds about 5mm in diameter and covered with red orange pulp.<sup>8</sup> The plant is used medicinally in Indo-China, the Philippines, Brazil, Guiana, Cambodia, North West Amazonia, Uruguay, West India, Central America and Venezuela. The dye obtained from the pulp of the seeds called bixin is used all over the world as a red orange dye for coloring rice, cheese, soft drinks, oil, butter and soup. The dye is also used in some regions to dye textiles and seeds are used as a condiment.<sup>9-11</sup> Various indigenous groups paint



their hair and bodies with the pulp to repel insects and protect from sunburn. The seeds are given to bulls to make them aggressive for bull fighters and are taken by Indians as an aphrodisiac.<sup>11</sup> In India, the plant is used by Ayurveda Practitioners as an astringent and mild purgative and is considered by them as a good remedy for treating dysentery and kidney diseases. The root bark is antiperiodic and antipyretic. 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 jaundice.<sup>12</sup>

The traditional healers claim that some medicinal plants such as *Bixa* sps; are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants represent an alternative treatment for non severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant.<sup>13</sup> The literature survey reveals that in traditional systems of medicine, different parts (leaves, seeds) of *Bixa*, a small tree seen throughout India, have been recommended for many more presumed beneficial uses and for the treatment of small burns, poisoning, thinning hair, headaches, gonorrhoea, skin diseases, nausea and vomiting, snake bites etc.<sup>14,15</sup> *Bixa* has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardio protective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions.



Figure 1: *Bixa orellana* linn

## MATERIALS AND METHODS

### Plant material Collection and Authentication

The fresh leaves of *Bixa orellana* L. were collected in the month of September 2012 from Marunje village, District - Pune (MS), India. and authentication was done by Dr.Pramod Patil ,Prof and Head, Department of Botany,Govt MLB girls PG College Bhopal (M.P) (Sheet no:339/5/2/2013. And collected fresh leaves were

washed and sun dried and used for the further investigations.

**Reagents:** All the reagents were of analytical grade.

### Methods

The organolaptic and morphological characters of the leaves were studied<sup>16</sup>, under dissecting microscope. Transverse sections were taken and studies<sup>17</sup>, for the different histological analysis along with the surface preparation. Whereas the dried powder material was studied for microscopical characters.<sup>18</sup> like stomata, trichome, lignified cells, palisade cells, parenchymatous cells, calcium oxalate crystals and xylem vessels. Powder drug was also studied with routine chemical reagents to observe the fluorescent effect under UV (Day light, Short wave length, and long wavelength). Physicochemical parameters were determined<sup>19</sup> like ash value, extractive value, loss on drying etc. Phytochemical constituents were identified by performing the preliminary phytochemical investigation<sup>20</sup> for different extracts. The extracts were prepared by using water, ethanol, methanol, chloroform and ethyl acetate. The results for all the parameters were illustrated and photographs of the microscopical characters were taken.

## RESULTS AND DISCUSSION

### Leaf morphology

Leaves spirally arranged, simple, stipulate, ovate, 7.5-24 x 4-16 cm, shallowly cordate to truncate at base, longly acuminate at apex, green or dark green above, grey or brownish-green beneath; scaly when young, glabrous; petiole, terete, thickened at both ends, 2.5-12 cm long.<sup>2</sup> shown in Figure 2 (a) and (b).



Figure 2a: Upper Surface of *Bixa orellana* Leaf

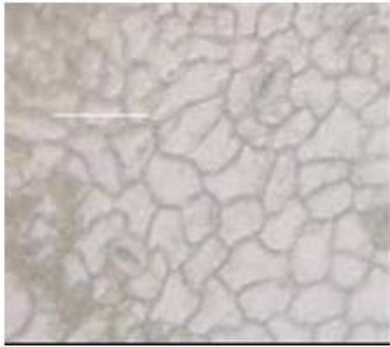


Figure 2b: Lower surface of *Bixa orellana* Leaf

**Microscopy**

**Surface preparation**

Leaf is dorsiventral shows the presence of anomocytic type of the stomata (Figure 03) covered with thin walled epidermal cells. Leaf surface also shows the presence of simple, multicellular, covering trichome and single layered epidermis covered with the cuticle.

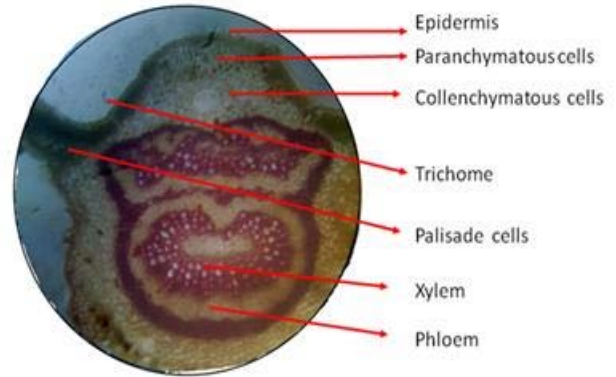


**Figure 3:** Stomata

**Transverse Section of Leaf**

The leaf is dorsiventral, T.S passing through the midrib region shows single layered epidermis on both the surfaces and covered with thick cuticle. Upper epidermis shows single layered having multicellular covering trichome. Lower epidermis is single layered elongated cell and closely arranged. Spongy parenchyma is multi layered, loosely arranged, round shaped and show pink coloured xylem vessels. Spongy parenchyma shows the

presence of calcium oxalate crystals. Transverse section passing through the laminar region shows bilayered, tubular, compactly arranged palisade cells just below the upper epidermis. Upper and lower epidermis followed by mesophyll consists of 6-8 layered parenchyma and 3-4 layered collenchyma. Meristeel consists of Vascular bundles which is concentric in shape shown in Figure 4.



**Figure 4:** T.S of midrib of *Bixa orellana* leaf with Lamina

**Powder analysis**

It is dark green, fine, odorless powder with slight bitter taste. The powder microscopy reveals the presence of multicellular trichome, anomocytic type of the stomata, calcium oxalate crystals, xylem vessels and phoem fibers. Powder shows the spiral thickenings vascular bundles, elongated palisade cells and thin walled parenchymatous cells, shown in Figure 5.



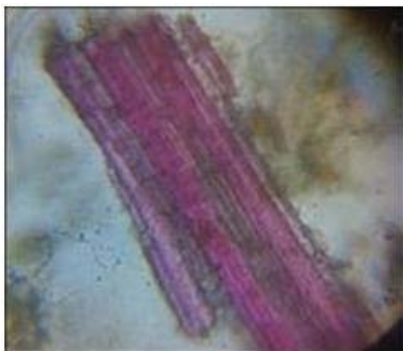
Trichome



Calcium oxalate crystal



Palisade cells



Phloem fibers



Spiral Vessels



Epidermal cells

**Figure 5:** Powder characters of *Bixa orellana* leaf

## Physico-chemical Parameters

### Description of leaf powder

Leaves were dried in sunlight and powder was prepared which shows colour. Same powder was subjected to various physicochemical reactions.

### Ash Value

Percentage of Total ash, Acid-insoluble ash water soluble ash values of the powdered drugs were performed as per Pharmacopoeial standard procedure and results were reported in Table 01.

### Extractive values

Water soluble and Alcohol soluble extractive values were determined according the Pharmacopoeial standard procedure and result were reported in Table 01.

### Moisture content determination

Moisture content determination was carried out by loss

on drying method according to the pharmacopoeial standard procedure and result were reported in Table 01.

**Table 1:** Physico-Chemical Evaluation of *Bixa orellana* Leaf

Physicochemical parameter	Results
Description	Green
Total ash	9% w/w
Water soluble ash value	2% w/w
Acid insoluble ash value	1.5% w/w
Water Extractive	7% w/w
Alcohol Extractive	9.5% w/w
Loss on drying	8% w/w

### Fluorescence Analysis of leaf powder<sup>21, 22</sup>

The powder was subject to fluorescence analysis with different acids and reagents. The behaviour of powdered drugs with different acid and chemical reagent was observed under UV light and visible light as per the standard procedure and result were reported in table 02.

**Table 2:** Fluorescence analysis of *Bixa orellana* leaf

Reagent	Colour of powdered crude drug		
	Long wavelength	Short wavelength	Day light
Powder as such	Dark green	Green	brown
Powder+ water	Dark green	Green	Yellowish brown
Saturated Picric acid	Dark green	Green	Brownish green
Powder + Conc HNO <sub>3</sub>	Brownish green	Yellowish brown	Yellowish brown
Powder + Conc HNO <sub>3</sub> (50%)	Dark green	Brown	brown
Powder + Conc H <sub>2</sub> SO <sub>4</sub>	Brown	Brownish green	Brownish black
Powder + Conc H <sub>2</sub> SO <sub>4</sub> (50%)	Greenish black	Green	Yellowish brown
Powder + Conc HCL	Green	Brownish green	Brown
Powder + Glacial Acetic Acid	Dark green	Dark green	Yellowish green
Powder + Iodine solution (N/20)	black	Dark green	blackish green
Powder+ FeCl <sub>3</sub> (5%)	Dark green	Dark green	Yellowish green
Powder+ NaOH (5%)	Dark yellowish brown	Yellowish brown	brown
Powder+ Ammonia solution	Dark green	Green	Yellowish brown
Powder+ KOH (5%)	Brown	Yellowish green	Yellowish brown

### Extraction of powdered leaves with different solvents

Extracts were prepared with various solvents like ethanol, methanol, ethyl acetate, chloroform and aqueous extract was prepared. Percentage yield of extract obtained were reported in Table 03.

**Table 3:** Extraction of *Bixa orellana* linn leaf with different solvents

Extracts (based on type of solvent used)	% w/w
Aqueous	7
Chloroform	1
Ethanol	9.5
Methanol	10.2
Ethyl acetate	2

### Preliminary Photochemical investigation

The ethanolic, methanolic, ethyl acetate, chloroform and aqueous extracts were investigated for the presence of preliminary phytoconstituents and results were reported in table 04.

### DISCUSSION

In the last two decades of the century the scientists are sincerely trying to evaluate many plant drugs used in traditional system of medicine. The pharmacognostical and phytochemical data is one the major criteria for identification of plant drug those supplied in crude as well as powdered form. It also helps to differentiate the closely related species or varieties with similar



pharmacology and chemical constituents. The leaves are greenish with a smooth and thick texture and possess number of trichomes on both leaf surfaces. The microscopy shows (fig 4) the presence of vascular bundle, collenchymas, spongy parenchyma and palisade cells. Palisade cells are elongated, bilayered, and tubular shaped and compactly arranged. Surface of the leaf show presence of simple multicellular covering trichome and anomocytic stomata (figure 3), while the powdered drugs shows the presence of trichomes, stomata, xylem vessels and phloem fibers, palisade cells, calcium oxalate crystals and epidermal cells. (figure 5)

The physicochemical constants such as total ash (9 %) and water soluble ash (2%), acid insoluble ash (1.5%), water extractive (7%) alcohol Extractive (9.5%) and loss on drying (8%) were determined (table 1). The soluble extractive value (table 3) with different solvents like

Ethanol (9.5%), Methanol (10.2%), Chloroform (1%), Ethyl acetate (2%), and aqueous extract (7%) and investigated for the types of chemical constituent present.

The behavioural changes of powder when treated with different acid and reagents were studied as fluorescence analysis. It shows different colors under daylight and UV (short and long wavelength) light. Various qualitative chemical test were carried out for the different extract which indicates the presence of Alkaloids, Tannins, Triterpenoids, Steroids, Sterols, Saponins, Flavones, Flavonoids. Thus the proposed work was aimed to design a perfect protocol for the identification of different species and result found to be significant and encouraging towards the goal for standardization. Further it provide a good platform to study its medicinal value in the treatment of diseases and for well being of the human health.

**Table 4:** Preliminary Phytochemical Investigation

Phytochemicals	Ethanol extract	Methanol Extract	Chloroform Extract	Aqueous Extract	Ethyl acetate Extract
Alkaloids	+	+	+	+	+
Glycosides	-	-	-	-	-
Anthraquinone glycosides	-	-	-	-	-
Gums mucilage	-	-	-	-	-
Proteins,	-	-	-	-	-
Amino acids	-	-	-	-	-
Tannins	+	+	+	+	+
Phenolic compound	-	-	-	-	-
Triterpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Sterols	+	+	+	+	+
Saponins	+	+	+	+	+
Flavones	+	+	+	+	+
Flavonoids	+	+	+	+	+
Thiol group	-	-	-	-	-

(+)= Present, (-) = absent

## CONCLUSION

The quantitative determination of pharmacognostic parameters is helpful in standardization for the crude drug. Physical and chemical evaluation of crude drug help in detection of adulteration and purity of the drug. Presence of many phytoconstituents may find use of this drug to cure some ailments:

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