



## Pharmacognostic Standardization, Physico and Phytochemical Evaluation of *Bixa orellana* linn. Seed

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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. It is considered as a good remedy for treating dysentery and kidney diseases. In present investigation, the detailed pharmacognostic study of *Bixa orellana* seed is carried out to lay down the standards which could be useful in future experimental studies. The study includes macroscopy, microscopy, preliminary phytochemical screening and physicochemical evaluation. In this study, the medicinal properties of *Bixa orellana* were evaluated by phytochemical screening of Aqueous, Chloroform, ethanol, methanol, ethyl acetate extract. In following extract Glycosides, Anthraquinone glycosides, Proteins, Amino acids, Tannins, Triterpenoids, Steroids, Saponins, Flavonoids were identified.

**Keywords:** *Bixa orellana*, Pharmacognosy, Microscopy, Phytochemical screening.

### INTRODUCTION

*Bixa orellana* L. commonly known as annatto 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> The plant grows equally well in low lands and mountainous regions or areas of higher lavations.<sup>2</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>3</sup> In India, the plant is cultivated and found wild especially in Western parts of the country. *Bixa orellana* is a shrubby tree which ranges from 3-10 meters in height. 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. Flowers are white or purplish white in color. 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>4</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 colouring 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 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>5-7</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 represents 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>8</sup>. The present study is aimed at the evaluation of the antibacterial potential of *Bixa orellana* using various organic solvents, with the hope that such a study will create an interest among the people to search for new phytodrugs.



Figure 1: *Bixa orellana* linn

## MATERIALS AND METHODS

### Plant material Collection and Authentication

The seeds 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. The seeds were dried in shade and stored at 25°C. It was powdered, passed through 40# and stored in air tight bottles. An exhaustive Pharmacognosy was carried out using standard methodology.<sup>9-20</sup>

### Macroscopic study

Capsules ovoid, dark red, seed smooth. Capsule subglobose or ovoid, slightly laterally compressed, 1.4- 2-4.5 cm, usually densely purple-brown spiny, rarely smooth; spines 1-2 cm. Seeds numerous, red-brown, obovoid-angular, 4-5 mm<sup>21</sup> shown in figure02.



Figure 2: Seeds of *Bixa orellana* Linn

### Physico-chemical parameters

#### 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 1.

#### Extractive values

Water soluble and Alcohol soluble extractive values were determined according to the pharmacopoeial standard procedure and results were reported in table 1.

#### 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 1.

Table 1: Physico-chemical evaluations of *Bixa orellana* seeds

S. No	Physicochemical parameter	Results
1	Description	Orange-red
2	Total ash	5% w/w
3	Water soluble ash value	2% w/w
4	Acid insoluble ash value	1.5% w/w
5	Water Extractive	13.5% w/w
6	Alcohol Extractive	8.5% w/w
7	Loss on drying	7% w/w

#### Fluorescence Analysis of Seed powder<sup>22, 23</sup>

The powder was subjected 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 2.

#### 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 3.

Table 2: Fluorescence analysis of seed powder

S. No	Reagent	Colour of powdered crude drug		
		Long wavelength	Short wavelength	Day light
1	Powder as such	Yellow brown	Brown	Brown
2	Powder+ water	Yellow brown	Brown	Brown
3	Saturated Picric acid	Reddish brown	Pinkish brown	Pinkish Brown
4	Powder + Conc HNO <sub>3</sub>	Dark brown	Brown	Brown
5	Powder + Conc H <sub>2</sub> SO <sub>4</sub>	Dark Brown	Whitish red	Red
6	Powder + Conc H <sub>2</sub> SO <sub>4</sub> (50%)	Dark brown	Red	Yellowish red
7	Powder + Conc HCL	Brown	Yellowish brown	Brown
8	Powder + Glacial Acetic Acid	Brown	Yellowish red	Reddish brown
9	Powder + Iodine solution (N/20)	Dark green	Dark green	Brownish black
10	Powder+ FeCl <sub>3</sub> (5%)	Dark green	Green	Yellowish brown
11	Powder+ KOH (5%)	Dark yellowish Brown	Yellowish brown	Brown

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

Extracts (based on type of solvent used)	% w/w
Aqueous	13.5
Chloroform	3.5
Ethanol	8.5
Methanol	9.5
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 4.

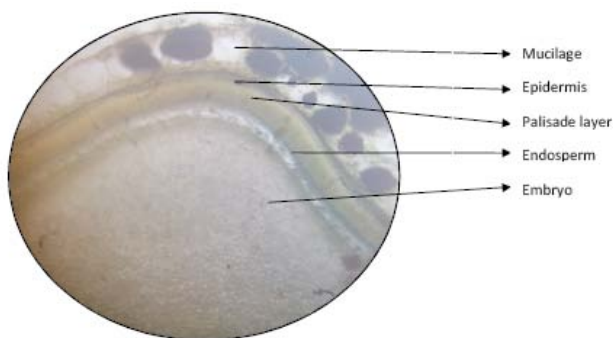
**Microscopy**

**Transverse section of *Bixa orellana* seed showed following features**

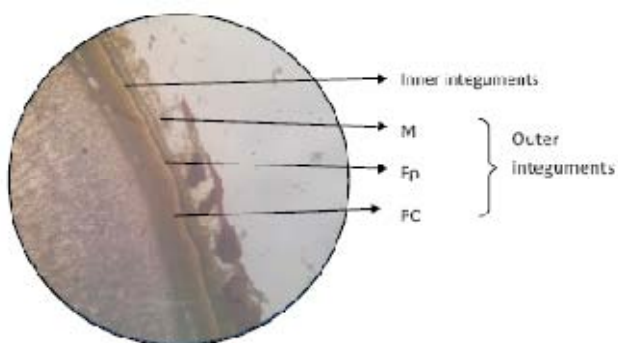
Epidermis: one to two layered epidermis. The transverse section of seeds revealed the presence of starch grains in the paranchymatous cell. The section of seed coat showed the inner and outer integuments and there is presence of palisade layer below epidermal layer (figure: 3).

**Table 4:** Preliminary phytochemical investigation of *Bixa orellana* seeds

Phytochemicals	Ethanol extract	Methanol Extract	Chloroform Extract	Aqueous Extract	Ethyl acetate Extract
Alkaloids	-	-	-	-	-
Glycosides	+	+	+	+	+
Anthraquinone glycosides	+	+	+	+	+
Proteins	+	+	+	+	+
Tannins	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Saponins	+	+	+	+	+
Flavonoids	+	+	+	+	+



**Figure 3:** T.S of Seed of *Bixa orellana*



**Figure 4:** TS of *Bixa orellana* seed showing outer and inner integuments

(M: mucilage, Ep: epidermis, Pc: Palisade cells)

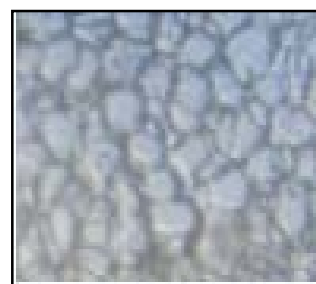
**Powder study of the *Bixa orellana* Seed powder**

Colour: Reddish Brown in colour

Parenchymatous cell: Oval shape parenchymatous cells are present.

Starch grain: small oval shape starch grains are present

Palisade layer: elongated palisade cells are present.



**Figure 5(a):** Parenchymatous cells



**Figure 5 (b):** Palisade cells



Figure5(c): Starch grains

## RESULTS AND DISCUSSION

The present study dealt with the pharmacognostic and phytochemical investigation of *Bixa orellana* seeds. Morphological and anatomical studies of the seed will enable to identify the crude drug. The information obtained from preliminary phytochemical screening will be useful in finding out the genuineness of the drug. Ash values, extractive values can be used as reliable aid for detecting adulteration. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production.

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