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Pharmacognostic and Phytochemical Investigation on Seed of *Bixa orellana* Linn

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ABSTRACT

According to ethanomedical information plant *Bixa orellana* Linn (Bixaceae), commonly known as "annatto" in English and "Sinduri" in Sanskrit. Annatto is mainly used for its bright red fruit (seedpods) as a natural colour and hence it's common name "Lipstick tree". It has been widely used to treat various ailments such as Gonorrhoea, inflammatory, mosquito repellent, haemostatic, anti-dysentric, diuretic, epilepsy, kidney and some skin diseases. It is commonly used as aphrodisiac medicine. It is also used to treat urinary difficulties and stomach problems**.** A non toxic- Annatto dye which is obtained from pulp is used for colouring edible materials. The unique red colour to annatto is due to bixin and norbixin, which are Carotenoids.

Present work was carried out to determine its macro morphological, and chemo-micro-morphological profiles. These findings will be useful towards establishing pharmacognostic standards on identification, purity and quality.

Keywords: *Bixa orellana* Linn, Pharmacognostic evaluation, Physicochemical Analysis.

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INTRODUCTION

Plants acquire curative properties or forth pharmacological effects on the animal body. Such plants are nominated as "Medicinal Plants" and serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine.[1] The medicinal value of plants lies in the active compounds which are chemically bioactive substances such as tannins, carbohydrates, terpenoids, steroids and flavonoids that generate definite physiological action on the human body.[2]

The growing demand for plant constituent in the cosmetic, food and pharmaceutical industries suggests that systematic profile studies of medicinal plants are ever more important in the drive to find active compounds for prospective applications.[3] Traditional medicine is an fundamental part of the health system in just beginning countries.[4] Medicinal plants play a key task in the world health care and about 80% of world is depend on plant medicine. There is the amplify need to search for potential drug-agent plants for the treatment of diseases, its aliment especially priority diseases in just developing countries such as HIV/AIDS, hypertension, sickle cell anaemia, diabetes and malaria.[5]

Bixa orellana L. (Bixaceae), commonly known as "annatto" in English and "Sinduri" in Sanskrit, is resident and native to tropical America but now cultivated in many tropical countries including India.[6,7] Annatto is mainly used for its bright red fruit (seedpods) as a natural colouring for food, textiles, objects, body, hairs and face hence it's common name "Lipstick tree".

Annatto seeds are astringent in taste, febrifuge in action and are used as remedy for Gonorrhoea. The pulp surrounding the seed is used as mosquito repellent, haemostatic, anti-dysentric, diuretic and is useful to treat epilepsy, kidney and some skin diseases. It is commonly used as aphrodisiac medicine, to treat inflammatory conditions and parasitic diseases. The decoction of leaves is used to prevent vomiting and nausea; to treat urinary difficulties and stomach problems.[8]

A non toxic - Annatto dye obtained from pulp is used for colouring edible materials such as butter, ghee, margarine, cheese and chocolate.[9]

Root as well as root bark is perspirent and anti pyretic in action which is used as antiperiodic and for controlling Asthmatic Paroxysm.[10, 11]

Bixin and norbixin are the two carotenoids which give annatto its unique red colour. It is a safe, economical and easy to handle product, among naturally occurring colorants is the second ranked in economic importance as it is the natural source of Bixin'with large s consumption value.[8, 12]

MATERIALS AND METHOD

Plant material Collection

The seeds of *Bixa orellana* L. were collected from Herbal Medicinal Garden of ADINA Institute of Pharmaceutical Sciences, Sagar; Madhya Pradesh, India in the month of September 2014 from ripens fruit. The seeds were dried in shade and stored at 30^0C. It was powdered, passed through 80# and stored in air tight container.

Chemicals

All the solvents and chemicals used were purchased from Merck chemical (LR grade), India.

Macroscopic Examination

The dried seed part of *Bixa orellana* was studied for its morphological characters such as colour, odour, shape, taste, dimension and hairs present or not, etc.

Figure 1: **Seeds of *Bixa orellana* Linn**

DETERMINATION OF PHYSICAL CONSTANTS PARAMETERS

Ash Value

Percentage of total ash, acid-insoluble ash and water soluble ash values of the powdered drugs were performed as per standard procedure mentioned in Indian pharmacopoeia.[13]

Extractive values

Water, Alcohol, Petroleum ether and Chloroform soluble extractive values were performed and determine as per standard procedure mentioned in Indian pharmacopoeia. [13]

Foreign Organic Matter and Moisture Content

Foreign organic matter was determined from the weight of the drug taken and moisture content was determined by loss on drying method in terms of percent w/w as per standard procedure mentioned in Indian Pharmacopoeia. [13]

Fluorescence Analysis of Seed powder

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 day light as per the standard procedure. [14]

Reaction of Powdered Drug with Different Reagents

Powdered drug was treated with different reagents and colours shown by that treatment is noted down. [14]

Qualitative Preliminary Photochemical investigation

The freshly prepared Aqueous, Hydro alcoholic and Chloroform extracts were investigated for the presence of preliminary phytoconstituents by using reported methods. [15, 16, 17]

QUANTITATIVE ESTIMATION OF PHYTO CONSTITUENTS

Estimation of Fat

3.0 g of powdered drug was dissolved in 100ml water, transfer to a separating funnel, acidified with sulphuric acid and extracted with successive quantities i.e. 50, 40, and 30 ml of ether, was mixed in a separating funnel and washed with water until the washings were free from mineral acid. The ether solution was transferred to a tarred flask; the ether was removed and dried the residue of fatty acids to constant weight at 80°.[18]

Estimation of Protein

The total protein content was estimated by Lowry method.[19]

Estimation of Carbohydrate

50 ml of alkaline cupric tartrate TS was pipetted into a 400ml beaker, 48ml of water was added, mixed. 2ml of above mixture that have been diluted quantitavely with water, upto 5.0% concentration. The solution was heated to boil, and continued boiling for 2 minutes. The hot solution was filtered through a tarred porcelain filtering crucible; the precipitate was washed with water maintained at 600C, then with 10 ml of alcohol. Dried at 105^0C to constant weight. A blank determination was performed, and made any necessary correction. The corrected weight for the precipitate was compared with dextrose (carbohydrate) of known concentration.[18]

Estimation of Total Flavonoids

Total flavonoid content in plant was estimated by spectrometric method. Dried powdered plant material (10 gm) was extracted by continuous mixing in 100 ml of 70% ethanol for 24 hr at room temperature. After filtration, ethanol was evaporated untill only water remained. Water phase was subsequently extracted with ethyl acetate. The extract was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum up to a concentration of 1gm/ml of extract. They were further diluted with ethyl acetate to obtain 0.01gm/ml solutions used in the experiments. About 10ml of the solution was transferred into a 25ml volumetric flask, to this 1 ml of 2% $AlCl_3$ was added and the solution was make upto 25ml with methanol-acetic acid and was kept aside for 30min, the absorbance was measured at 390nm against the same solution without $AlCl_3$ being blank. Luteolin was used to construct the

calibration curve in the concentration range 1.0-10.0 µg/ml. Result was calculated by using caliberation curve method. [20]

RESULTS AND DISCUSSION

Macroscopic Evaluation

Table 1: Macroscopic Evaluation of *Bixa orellana seeds*

Evaluation Parameter	Results
Colour	Dark Red
Odour	Odourless
Taste	Astringent
Shape	obovoid-angular
Size	4-5 mm
Facture	Smooth pulpy

Determination of Physical Constants

Table 2: Ash value of *Bixa orellana seeds*

S.N	Ash Value	Yield (% W / W)
1	Total ash	6.55
2	Water- soluble ash	2.69
3	Acid insoluble ash	1.58

Table 3: Extractive value of *Bixa orellana seeds*

S.N	Extractive value	Yield (% W / W)
1	Water extractive	21.5
2	Alcohol extractive	13.6
3	Petroleum ether extractive	9.7
4	Chloroform extractive	7.5

Table 4: Foreign Organic Matter and Moisture Content of *Bixa orellana seeds*

S.N	Particulars	Yield (% w / w)
1	Foreign Organic Matter	2.5
2	Moisture Content	15.82

Table 5. Fluorescence Analysis of Bixa orellana seeds

S.N.	Reagents	Day light	UV Light
1.	Drug Powder	Brown	Yellow brown
2.	Drug Powder +1M NaOH	Yellowish Brown	Brown
3.	Saturated Picric acid	Pinkish Brown	Reddish brown
4.	Drug powder + HCL	Reddish brown	Dark brown
5.	Drug Powder + 50 % nitric acid	Brown	Dark brown
6.	Drug powder + 5% $FeCl_3$	Yellowish Brown	Dark Green
7.	Drug powder +80 % H_2SO_4	Yellow Red	Dark Brown
8.	Drug powder + Water	Brown	Yellow brown
9.	Drug powder+Conc.H_2SO_4	Red	Dark Brown
10.	Powder + Glacial Acetic Acid	Reddish Brown	Brown

Table 5: Behaviour of the Powder of Bixa orellana seeds with Different Chemical Reagents

S.N	Treatment	Colour
1	Powder as such	Orange brown
2	Powder + Conc. sulphuric acid	Red
3	Powder + Conc. nitric acid	Brownish red
4	Powder + Conc. Hydrochloric acid	Reddish brown
5	Powder + 5% I2	Brownish black
6	Powder + 5N NaOH	Yellowish Brown
7	Powder + glacial Acetic acid	Reddish Brown
8	Powder + 80% H2SO4	Yellow Red

(Aq= Aqueous; HA= Hydro alcoholic; C= Chloroform)

Table 6: Preliminary Photochemical investigation of *Bixa orellana seeds*

SN	Phytochemical tests	Aq	HA	C
1	Alkaloid	+	+	+
2	Glycoside	-	-	-
3	Tannin	+	+	+
4	Saponin	+	+	+
5	Steroid	+	+	+
6	Flavonoid	-	+	+
7	Protein	-	+	-
8	Phenol	+	-	-
9	Gum mucilage	-	-	-

Quantitative Estimation of Phyto Constituents

Table 7: Quantitative estimation of Phyto constituents of *Bixa orellana seeds* Each 100 grams of sample contains

S.N	Phytoconstituents	% w/w
1.	Fat	5.48
2	Protein	7.22
3.	Carbohydrate	8.53
4.	Total flavanoids	6.5

CONCLUSION

In conclusion, the present study is related to pharmacognostical and phytochemical account of *Bixa orellana seeds* provided useful information in regard to its correct identity and evaluation, and helps to differentiate from the closely related other species of *Bixa orellana*, Linn. The other parameters observed are also useful for the future identification of the plant, and serves as a standard monograph for identification and evaluation of plant.

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