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Pharmacognostical Studies, Physicochemical Analysis and Evaluation of Laxative Activity of Various Leaf Extracts of *Bixa orellana* Linn.

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ABSTRACT

Bixa orellana is an important medicinal plant of the family (Bixaceae). it is highly valued from time immemorial because of its vast medicinal properties. The present work deals with the pharmacognostical studies, physicochemical analysis & laxative activity of various extracts of B. orellana leaves .Laxative activity was checked by using Wister strain albino rats . All the crude extracts such as ethanol, ethyl acetate, methanol and petroleum ether were tested for laxative(200&400 mg/kg).Where agar-agar used as a standard drugs. The preliminary phytochemical screening showed that the different solvent extracts of B. orellana contains alkaloids, flavonoids, terpenoids, saponins, glycoside, steroids and tannins. From the pharmacognostical studies and physicochemical analysis of leaves of B. orellana , it revealed a set of parameters which may enable to those who handle this plant to maintain its quality control, Adulteration and substitution relating to genuineness of drug. The extracts was found to produce significant laxative activity in dose dependant manner. The ethyl acetate extract was found to possess the most effective laxative activity.

Keywords: *Bixa orellana*; Acute toxicity study ; Laxative activity ; agar-agar

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INTRODUCTION

The tribal areas of Koraput (District) of Eastern Orissa(India) due to its unique varieties geographical and climatic factors have had a rich variety of medicinal plant *Bixa orellena* also known as sindur (Oriya) were frequently distributed and extensively used traditionally by the tribal people for curing their aliments. *Bixa orellana* L. (Bixaceae), commonly known as annatto in English , Sinduri‘ in Sanskrit and sindur in Odia is indigenous and native to tropical America but now cultivated in many tropical countries including India.[1-3]. *Bixa orellena* is an evergreen shrub or small tree, 2-8 m high bark light to dark brown, tough, smooth, sometimes. Leaves spirally arranged, simple, stipulate, ovate, 7.5-24 x 4-16 cm, shallowly cordate to truncate at base, longley acuminate at apex, green or dark green above, grey or brownish. Flowers in terminal branched panicles, 8-50 flowered, covered with reddishbrown scales; petals 4-7, obovate, 2-3 x 1-2 cm, pinkish, whitish . Fruit a spherical or broadly elongated ovoid capsule, 2-4 x 2-3.5 cm, flattened, green, greenish-brown or red when mature; seeds numerous, with bright orange-red fleshy coats[4-6]. Traditionally the plant was used as a colouring agent, it is also used to colour butter, cheese, beverages and fish and meat products . it has been used as an ingredient in weight- loss products and also in the treatment of snakebite. It is also used in the formation of herbal lipstick. Annatto possesses various pharmacological activities like anti-diarrheal, anti- inflammatory, antioxidant, hypoglycemic, anti- bacterial. *B. orellana is* known to have bioactivity, particularly regarding seed and leaf extracts. Scientific evidences show that it possesses antioxidant, antimicrobial, anticonvulsant, antidiabetic and cardio-protective activity[7-10]. The decoction of leaves is used to prevent vomiting and nausea; to treat urinary difficulties and stomach problems.[11]. Roots and

leaves of the plant are useful for the treatment of sore throat, jaundice, snake bites, dysentery ,gonorrhea, liver disease, diuretic and antipyretic agent including malaria [12]. The tree was incorporated into the traditional medicine of India, where different parts of the plant are used as diuretic, laxative, antibilious, antiemetic, and astringent agents, as a blood purifier, in jaundice, in dysentery, diabetes, diarrohea, fever, fluid retension[13,14].

MATERIALS AND METHODS

Drugs and chemicals

Agar-agar was procured from Bangalore Fine Chem. , Bengalur, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. Methanol GR 80°C, petroleum ether AR 40-60°C from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals reagents used in present work were procured from authorized dealer.

Collection of Plant Material

The leaves of *Bixa orellana* were collected from the Herbal garden of Jeypore college of pharmacy, Jeypore, Koraput district.(India) in the month of December 2015.The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-305/15,dated (7.12.2015). After authentification leaves were collected in bulk and washed under running tap water to remove adhering dirt. Then the leaves were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder. and stored in a closed air tight container for further use.

Pharmacognostical studies

Macroscopy study

Leaves spirally arranged, simple, stipulate, ovate, 7.5-24 x4-16 cm, shallowly cordate to truncateat 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.

Quantitative microscopy

The important identifying characteristic of leaf constants like Stomatal Number, Stomatal Index, Vein-islet number, Vein termination number were found out.[15,16].

stomatal number and stomatal index

It is the average number of stomata per square mm of the epidermis of the leaf. Stomatalindex is the percentage which the number of stomata forms to the total number of epidermal cells, each stomata being counted as one cell. Stomatal index can be calculated by using following equation.

$$I = \frac{S}{E + S} \times 100$$

I = Stomatal index , S = No. of stomata per unit area , E = No. of epidermal cells in the same unit area.

Tableno:1 Determination of stomatal number and stomatal index of *B.orelana* leaf

Part used	Stomatal number/mm^2	Epidermal cell/ mm^2	Stomatal index
Lower epidermis	12	102	10.5
	10	106	8.6
	11	109	9.1
	12	103	10.4
	9	101	8.1
Upper epidermis	13	102	11.3
	15	105	12.5
	17	104	15.3
	11	103	9.6
	14	106	11.6

Lower epidermis : Average stomatal number- 10.8

Average stomatal index-9.34

Upper epidermis: Average stomatal number- 14.0
Average stomatal index- 12.6

Palisade ratio

The Average no. of palisade cell beneath each epidermal cell.

Tableno :2 Determination of Palisade ratio of *B.orellana*

Sl no.	Epidermal cells	Palisade cells			Palisade ratio		
		Base	Middle	Apex	Base	Middle	Apex
1	4	22	25	23	5.5	6.25	5.75
2	4	24	24	20	6.0	6.0	5.0
3	4	23	22	20	5.75	5.5	5.0
4	4	26	20	22	6.4	5.0	5.5
5	4	22	23	21	5.5	5.75	5.25

Average palisade ratio:

Base : 5.83
Middle : 5.7
Apex : 5.3

Vein islet number

A vein-islet is the small area of green tissue surrounded by the veinlets. The vein-islet number is the average number of vein-islets per square millimeter of a leaf surface. It is determined by counting the number of vein-islets in area of 4 sq. mm. of the central part of the leaf between the midrib and the margin.

Tableno: 3 Determination of Vein islet number of *B.orellana* leaf

Sl no.	No. of Vein islet per mm^2
1	8
2	10
3	7
4	9
5	8

Average Vein islet no. : 8.4

Vein termination number

Veinlet termination number is defined as the number of veinlet termination per sq. mm of the leaf surface, midway between midrib of the leaf and its margin. A vein termination is the ultimate free termination of veinlet.

Tableno: 4 Determination of Vein termination number of *B.orellana* leaf

Sl no.	No. of Vein termination per mm^2
1	11
2	12
3	9
4	10
5	9

Average Vein termination no. : 10.2

Preparation of Extracts

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, methanol and petroleum ether as solvent. A total amount of 650 g coarse powder was extracted with 1200 ml of each solvent. For each solvent,10 cycles were run to obtain a thick slurry. Each slurry was then concentrated under reduced pressure to obtain the crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study[17].

Preliminary phytochemical investigation

The crude ethanol, ethyl acetate, methanol and pet. ether extracts of the leaf of *Bixa orellana* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the chemical analysis[17,18].

Table 5: Phytochemical screening for the different solvent extracts of of *Bixa orellana* leave

Extracts	Phytochemicals							
	Alkaloids	Flavonoids	Steroids	Glycoside	Carbohydrate	Tannins	Saponins	Terpenoid
Ethanol	++	+++	+	++	--	+ ++	+++	+
Ethyl-acetate	+	+	+	+	--	+	+	+
Methanol	+	++	+	++	--	++	+ +	+
Petroleum ether	--	+	--	+	--	+	--	--

+++, Strong; ++, moderately; +, poor presence ; -- , absenc

Physicochemical Analysis:

Different physicochemical values such as Fluorescence analysis, extractive values, ash values(total ash, acid-insoluble ash & water soluble ash), loss on drying, Foreign organic matter of *Bixa orellana* leave were determined according to the standard methods [18].

Fluorescence analysis:

The fluorescence analysis of the drug helps to identify the drug with specific fluorescent colours, and also to find out the fluorescent impurities. Thus the study of fluorescence analysis can be used as a diagnostic tool for testing adulteration[19].

Table no 6 : Fluorescence analysis of *Bixa orellana* leave powder

Solvents Used	Observation		
	Day Light	UV 254nm	UV 366nm
Drug powder	Green	Light green	Dark green
Petroleum ether	Grayish yellow	Orange red	Orange red
Ethanol	Grayish yellow	Orange red	Dark Orange red
Ethyl acetate	Grayish yellow	Orange red	Dark Orange red
Conc. H2SO4	Brownish black	Brown	Brownish green
50% H2SO4	Light brown	Brown	Dark brown
Conc.HNO3	Yellowish brown	Yellowish brown	Dark Yellowish brown
Conc.HCl	Light Green	Golden yellow	Dark brown
NaOH 5%	Light brown	Yellowish Brown	Dark yellowish Brown

Table no 7: Determination of Extractive value of *Bixa orellana* leave powder

Analysis parameters	Value (% w/w)
Alcohol soluble extractives	9.2 ± 0.65
Water soluble extractives	6.22±0.41
Ether soluble extractives	1.2±0.24

Results expressed as, Mean±SD, n=3

Table no 8: Determination of ash value of *Bixa orellana* leave powder

Analysis parameters	Value (% w/w)
Total ash	9.2 ± 0.21
Acid insoluble ash	1.3± 0.11
Water soluble ash	2.1 ±0.71
Sulphated ash	0.8±0.12

Results expressed as, Mean±SD, n=3

Table no 9: Determination of Moisture Content And Foreign organic matter of *Bixa orellana* leave powder

Analysis parameters	Value (% w/w)
Moisture Content	9 ±0.31
Foreign organic matter	1.3±0.24

Results expressed as, Mean±SD, n=3

Acute toxicity studies

The acute toxicity was performed according to OECD 423, 2001.The selected female albino rats were used to determine the dose. The animals were divided into twelve groups of three in each.The animals were fasted overnight prior to the acute experimental procedure. Distilled water was used as vehicle to suspend the different leave extracts of *B.orellana* and administered orally as following doses of 100, 300,600,1000 and 2000 mg/kg body weight. Immediately after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24hrs and daily for 14 days respectively [20].

Laxative activity

Animals

Healthy Wister strain albino rats were used.They were housed in standard conditions of temperature (25±2 °C), 12 hours light per day cycle, relative humidity of 45-55 % in animal house of Jeypore College of Pharmacy. They were fed with standard pellets of food and water. Animals were kept and all operation on animals was done in aseptic condition.

Drugs

Agar-agar (300 mg/kg,p.o.) of different *Bixa orellana leave* extracts used for activity study. The doses were prepared either with vehicle (1% Tween-80 solution in normal saline or normal saline were administered orally

Experimental protocol

Animals were selected, weighed (25-30 g) and devided in to ten groups (n=6), namely control, standard drug and four groups belonging to four different extract of *B.orellana*. All the studies conducted were approved by the Institutional Animal Ethical Committee (1200/ac/08/CPCSEA), Dadhichi college of pharmacy, Vidya vihar,Cuttack, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Procedure

The test was performed according to Capasso et. al.on rats of either sex weighing 200-220 g were kept in individual cages during one week. Any rat producing wet feces was rejected. The rats were fasted for 12 h before the experiment, but with water provided ad libitum. The animals were divided into eight groups of six in each. The animal groups were administered orally either with vehicle (1% Tween-80 solution in normal saline, 25(ml/Kg), reference standard drug, agar-agar (300 mg/ kg, p.o.) in saline or doses of extract (200 and 400mg/kg) .Immediately after dosing, the animals were separately placed in cages suitable for collection of faeces. (each cage is with a wire mesh at the bottom and a funnel to the urine; stainless-steel sieves are placed in the funnel to retain feces). After 8h of drug administration, the faeces were collected and weighed.Thereafter, food and water were given to all rats and faecal outputs were again weighed after a period of 16 h.[21,22].

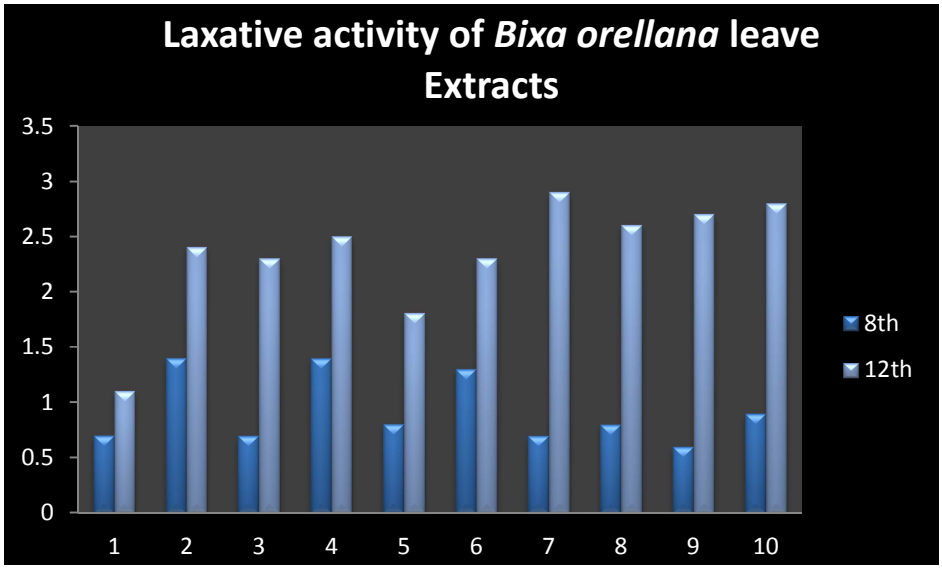
Table no 10 : Laxative activity of various leave extracts of *Bixa orellana*

Treatment	Dose mg/kg	Fecal out put(g)	
		8 hours	8-16 hours
Control	-----	0.7 ± 0.3	1.1 ± 0.6
Agar-Agar (standard)	300	1.4 ± 0.2	2.4 ± 0.1
Ethanol extract	200	0.7± 0.3	2.3 ± 0.2
	400	1.4± 0.3	2.5 ± 0.1
Ethyl acetate extract	200	0.8 ± 0.1	1.8 ± 0.2
	400	1.3± 0.2	2.3 ± 0.1
Methanol extract	200	0.7 ± 0.2	2.9 ± 0.3
	400	0.8 ± 0.3	2.6 ± 0.1
Pet. ether extract	200	0.6 ± 0.1	2.7 ± 0.2
	400	0.9 ± 0.3	2.8 ± 0.3

Statistical analysis

The data are represented as mean ± SEM, and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey post test where $P < 0.05$ was considered statistically significant.[23].

Fig no 1: Laxative activity of various leave extracts of *Bixa orellana*



1: Normal control, 2: Standard (Agar-agar), 3:Ethanol ext.200mg/kg ,4:Ethanol ext.400mg/kg , 5:.Ethyl acetate ext.200mg/kg , 6:Ethyl acetate ext.400mg/kg , 7 :Methanol ext.200mg/kg , 8:Methanol ext.400mg/kg , 9: Pet.ether ext.200mg/kg ,10:Pet.ether ext.400mg/kg

RESULTS AND DISCUSSION

The quantitative microscopic study was carried out on the leaves of *B.orellana* and obtained the following results.The average stomatal number and the stomatal index of lower epidermis & the upper epidermis of the leaf shown in(Table no.1)The result shows the upper epidermis of the leaf contain more stomatal number than lower epidermis .The average palisade ratio of the leaf shown in(Table no.2) From the study it is observed that the apex of leaf is having lower palisade cells as compared to base and middle part of the leaf. The vein islet number and veinlet termination number of *B.orellana* found to have 8.4 and 10.2 shown in(Table no.3&4) . The preliminary phytochemical screening showed that the different solvent extracts of *B. orellana* contains alkaloids, flavonoids, terpenoids, saponins, glycoside, steroids and tannins in all the solvent extracts & carbohydrates absent in all the extracts. The ethanol extract yielded strongly, all the phytochemicals followed by petroleum ether,methanol and ethyl acetate. The pet.ether extract also yielded only flavonoid,glycoside and tannin at the poor presence which were shown in (Table no. 5) .The Physicochemical analysis of the plant material such as the extractive value, ash value, loss on drying and fluorescent analysis of the leaf powder shown in (Table no.7,8 & 9). The Percentages of the extractive values were calculated with reference to air-dried drug. The extractive values in different solvents indicate the amount and nature of constituents in the extracts. The extractive values were also helpful in estimation of specific constituents soluble in particular solvent. The fluorescence analysis of the powdered drug of *B.orellana* in various solvents was performed under normal and UV light to detect the fluorescent compounds. The fluorescence analysis of the drug helps to identify the drug with specific fluorescent colours, and also to find out the fluorescent impurities. Thus the study of fluorescence analysis could be used as a diagnostic tool for testing adulteration result were shown in (Table no. 6). The extracts was found to produce significant laxative in dose dependant manner. The ethyl acetate extract was found to possess the most effective laxative activity as compared to standard drug Agar-agarl in a dose of (300mg/kg).which are shown in (Table no. 10) and graphically represent in (Fig. no.1).

CONCLUSION

This study on the pharmacognostical and phytochemical analysis of leaves of *B. orellana* revealed a set of parameters which may enable to those who handle this plant to maintain its quality control. Adulteration and substitution have become a major problem due to the absence of standards relating to genuineness of drug. On the basis of the present study, we may conclude that *Bixa orellana* leave produces significant laxative activities in

dose-dependent manner on animal models., The traditional use has been pharmacologically validated. Since, *Bixa orellana* leave showed remarkable activity when compared with standard drugs. Therefore, *Bixa orellana* leave can be a substitute of synthetic laxative drugs having no adverse effects.

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