APJNP 2016; Vol.1 (01): pp. 47-54 **Original Research Article**

ASIAN PACIFIC JOURNAL OF NATURAL PRODUCTS

Available online at http://ainstin.com

Received: 24-08-2016
Revised: 03-09-2016
Accepted: 05-09-2016

Subhashree Padhi
Jeypore College of Pharmacy,
Rondapalli, Jeypore – 764002,
Koraput, Odisha,India

Sangram. K .Panda
Jeypore College of Pharmacy,
Rondapalli, Jeypore – 764002,
Koraput, Odisha,India

Corresponding Author
Sangram. K .Panda
Email:
sangrampanda2009@gmail.com

EVALUATION OF ANTIPYRETIC AND ANTIDIARRHOEAL ACTIVITY OF VARIOUS LEAF EXTRACTS OF *BIXA ORELLANA LINN*.

Subhashree Padhi and Sangram. K .Panda

ABSTRACT

Bixa orellana is an important medicinal plant of the family (Bixaceae) and it is highly valued from time immemorial because of its vast medicinal properties. The present work deals with the investigation of antipyretic and antidiarrhoeal activity of various extracts of *Bixa orellana* leaves. Antipyretic and antidiarrhoeal activity was screened in using Wister strain albino rats and mice. All the crude extracts such as ethanol, ethyl acetate, methanol and petroleum ether were tested for antipyretic activity at 100 and 200mg/kg & antidiarrhoeal activity at 200 and 400 mg/kg by using Paracetamol (100 mg/kg) & loperamide (3 mg/kg)) as standard drugs respectively.The extract was found to produce significant antipyretic & antidiarrhoeal activity in dose dependant manner. The ethanol & ethyl acetate extract in a dose of 200 mg/Kg body weight exhibited significant antipyretic activity after 90 minutes and 120 minutes as compared to standard drug. Whereas the ethanol & methanol extracts were found to be effective against castor oil induced diarrhoea on experimental mice at the dose of 400 mg/kg body weight.

Keywords*:* *Bixa orellana,* antidiarrhoeal, antipyretic activity

INTRODUCTION

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects [1]. In developing countries like India, a majority of people who live in the rural areas almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhoea [2]. It is necessary to establish the scientific

Basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs.

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 ailments. *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.[3-5].

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 reddish brown 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 [6-8]. 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 [9-12]. The decoction of leaves is used to prevent vomiting and nausea; to treat urinary difficulties and stomach problems.[13]. 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 [14].

MATERIALS AND METHODS

Drugs and chemicals

Paracetamol & Loperamide was procured as gift sample from Provizer Pharma, Surat, Gujarat, India & Taj Pharmaceuticals Ltd,Mumbai,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,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.

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 [15,16].

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 phyto-constituents by carrying out the chemical analysis[16,17].

Table no 1: Phytochemical screening for the different solvent extracts of *Bixa orellana* leaves

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

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

Evaluation of Antipyretic 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.

Experimental protocol

Animals were selected, weighed (25-30 g) and divided 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.

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 [18].

Antipyretic activity by Yeast induced pyrexia

For studying antipyretic activity of, albino rats weighing150-200 gms were selected and divided into ten groups containing six animals in each group were used for yeast induced pyrexia models**.** Group I animals received 1 ml/kg body weight of normal saline orally and served as control group. Group II animals were treated with paracetamol by intraperitoneal injection in the dose of 100 mg/kg body weight and served as standard group. The animals of group 3 two 10 received the ethanol, ethyl acetate, methanol and petroleum ether of root extract of *B.orellana* orally (100&200mg/kg body weight) to the respective groups of animals. In the beginning of the experiment normal rectal temperatures was noted by inserting 2cms of digital thermometer, lubricated with glycerine into the rectum. Pyrexia was induced by intraperitonial injection of 2ml/kg body weight of 15% brewer's yeast suspension in normal saline. The animals were then fasted for the duration of experiment (approximately 24 hours). After 18 hours of yeast injection, extracts (100&200 mg/kg body weight) are given to the respective test group animals then the basal temperatures were recorded for all the groups of animals by inserting 2cms of digital thermometer, lubricated with glycerin into the rectum. The rectal temperatures of all the animals were noted at 30 minutes of intervals till 3 hours. [19,20].

Table no 2: Effect of various leaf extracts of *B.orellana* against yeast induced pyrexia in rats

Group	Treatment Dose (mg/Kg)	Initial Body Temperature (ºC)	Basal Temperature (ºC)	30min	60min	90min	120min	180min
Control	-------	38.36±0.08	38.33±0.6	39.47±0.12	39.38±0.14	39.40±0.12	39.52±0.07	39.02±0.14
Paracetamol (standard)	100	37.35±0.07	39.12±0.14	38.31±0.07	38.27±0.10	37.42±0.06	37.18±0.11	37.21±0.13
Ethanol extract	100	37.42±0.07	39.22±0.21	38.35±0.23	38.27±0.14	38.53±0.18	39.42±0.07	39.24±0.17
	200	37.36±0.14	39.61±0.16	37.41±0.12	38.45±0.18	39.33±0.11	39.32±0.15	39.26±0.04
Ethyl acetate extract	100	37.34±0.06	38.48±0.13	38.73±0.18	38.42±0.08	39.27±0.21	38.32±0.31	38.24±0.23
	200	37.51±0.26	37.26 ±0.11	39.47±0.25	38.24±0.05	39.31±0.23	39.20±0.11	39.42±0.16
Methanol extract	100	37.51±0.17	37.24±0.05	38.26±0.12	37.27±0.18	38.44±0.09	38.20±0.22	39.43±0.21
	200	37.26± 0.6	37.43 ± 0.11	37.52±0.27	38.40±0.07	37.33±0.14	39.47±0.18	38.28±0.07
Pet. ether extract	100	37.45±0.22	38.40±0.26	37.32±0.28	38.26±0.06	38.61±0.11	39.51±0.13	38.23±0.18
	200	37.31±0.09	37.34±0.18	37.57±0.16	38.22±0.05	39.36±0.22	38.41±0.11	39.53±0.17

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 $P < 0.05$ as significant.

Evaluation of Antidiarrhoea Activity

Animals

Healthy albino mice of Swiss strain of either sex 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

Loperamide (3 mg/kg) and a dose of 200 & 400 mg/kg of different *B.orellana leaf* extracts used for activity study and the route of administration for both standard and test drug was orally.

Castor oil induced diarrhea

The animals were all screened initially by giving 0.5 ml of castor oil one week before the actual experiment. Only those showing diarrhoea were selected for the final experiment. Thirty mice fasted for 24 h were randomly allocated to five groups of five animals each. Group I (received 1% tween 80 at a dose of 10 ml/kg) served as control group, Group II received the standard drug loperamide 3 mg/kg, p.o. Group III to X received the different leaf extracts of *B.orellana* at the doses of 200 and 400 mg/kg p.o., respectively. One hour after administration, all animals received 0.5 ml of castor oil and then they were individually place in cages the floor of which was lined with transparent paper. During an observation period of 4 h, the time of onset of diarrhoea, the total number of faecal output (frequency of defecation) and weight of faeces excreted by the animals were recorded [21]

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 [22].

Table no. 3. Effect of *B.orellana* leaf extract on castor oil induced diarrhea in mice

Group	Treatment Dose (mg/Kg)	Time of onset of diarrhoea(min.)	Total number of faeces in 4h(frequency of defecation in 4 h)	% Inhibition of defecation	Weight of stool(g)
Control	----------	87 ± 12.2	8.1 ± 1.4	---------	0.87 ± 0.07
Loperamide	3	247.3 ± 6.0	1±0.3	71.67	0.06 ± 0.02
Ethanol extract	200	172.2 ± 14	3.4 ± 0.3	57.73	0.27 ± 0.02
	400	223.4 ± 11.6	1.6 ± 0.2	73.84	0.03 ± 0.04
Ethyl acetate extract	200	167.7±24.3	4.2±0.4	48.31	0.21±0.014
	400	173.7±12.4	8.3±0.2	66.41	0.31±0.027
Methanol extract	200	163.2±16	3.8±0.4	51.68	0.23±0.024
	400	217 ±11	1.8±0.3	67.32	0.24±0.021
Pet. ether extract	200	157.2±7	7.2±0.9	41.18	0.22±0.02
	400	187.2±16	6.7±0.3	37.21	0.16±0.012

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 $P < 0.05$.

RESULT AND DISCUSSION

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. 1). A preliminary acute toxicity study in mice showed that all the extracts were not toxic (LD50 > 2000mg/kg). Among all the extracts ethanol &ethyl acetate shows significant antipyretic activity effect than other two extract in a dose of 200 mg/kg body weight as compared to standard drug paracetamol in a dose of (100mg/kg) which were shown in (Table no. 2) .Castor oil induced diarrhoea on experimental mice at the dose of 400 mg/kg body weight, The extract produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation and consistency of faeces in albino mice. At the same dose, the extract showed significant antidiarrhoeal activity showing 73.84 % &67.32 % (ethanol & methanol) extracts reduction in diarrhoea comparable to that of the standard drug loperamide that showed 71..67% reduction in diarrhea (Table No.3).

CONCLUSION

On the basis of present study, we may conclude that *Bixa orellana* leaf extracts produces significant antipyretic and anti-diarrhoeal activities in dose-dependent manner on animal models. By the positive activity of *Bixa orellana* leaf against

pyrexia,The traditional use has been pharmacologically validated. It all so showed significant anti-diarrhoea activity as compared to reference drug loperamide. The folklore claim of *Bixa orellana* leaf used as an anti-diarrhoeal have been confirmed .The preliminary phytochemical screening of *Bixa orellana* leaf extracts showed the presence of alkaloids, tannins, flavonoids, terpenoids, steroids and saponins.These constituents may be responsible for the *in vivo* anti-diarrhoea activity.Further studies to isolate and reveal the active compound present in the crude extract of *Bixa orellana* leaf and to establish the MOA of anti-diarrhoeal activity.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgment

We are thankful to Jeypore College of Pharmacy authority and all department of this college for good co-operation during this research work and also thank to local people of south eastern Odisha and the Biju Patnaik Medicinal Plants Garden and Research Centre, Jeypore, Koraput (District), Orissa, India. for providing valuable information about the plant.

REFERENCES

1 . U. N. Brahmachari. *Current Science*, 2001, 81(1), 15-16.

2. Syder JD, Merson MH. The magnitude of the global problem of acute diarrhoeal disease; a review of the active surveillance data. *Bulletin of the World Health Organization* 1982; 60:605-13.

3. Kirtikar KR, Basu BD. Indian medicinal plants. Periodical Experts Books Agency, Delhi 1935; 4(2): 2423-36.

4. Cooke T. The Flora of Presidency of Bombay. Botanical Survey of India, Calcutta . Vol. II. 1967.

5. Naik VN. Flora of Marathwada. Amrut Prakashan, Aurangabad, Vol. 1, 1998.

6.Anon. The cultivation of annatto. Farmer Jamaica 1962; 67(5):156-158.

7.Anon. The useful plants of India. Publications & Information Directorate, CSIR, New Delhi, India 1986.

8.Dendy DAV. Annatto the pigment of Bixa orellana. East African Agriculture and Forestry Journal 1996;32(2):26-132.

9. Patnaik S, Mishra SR and Choudhury GB. Phytochemical investigation and simultaneously study on anticonvulsant, antidiabetic activity of different leafy extracts of *Bixa orellana* Linn. International Journal of Pharmaceutical and Biological Archives 2011; 2(5): 1497–1501.

10. Selvi AT, Dinesh MG, Satyanet RS, Chandrasekaran B and Rose C. Leaf and seed extracts of *Bixa orellana* L. exert anti-microbial activity against bacterial pathogens. Journal of Applied Pharmaceutical Science 2011; 1(9): 116–120.

11. Asokkumar K, Jagannath P, Umamaheswari M, Sivashanmugam T, Subhadradevi V and Madeswaran A. Cardioprotective activity of *Bixa orellana* L. on isoproterenol induced myocardial necrosis in rats. Journal of Pharmacy Research 2012; 5(4): 1930–1934.

12. Viuda-Martos M, Ciro-Gomez GL and Ruiz-Navajas Y. *In vitro* antioxidant and antibacterial activities of extracts from annatto (*Bixaorellana* L.) leaves and seeds. Journal of Food Safety 2012; 32(4): 399–406.

13. Giorgi A, De Marinis P, Granelli G, Chiesa LM and Panseri S. Secondary Metabolite Profile, Antioxidant Capacity, and Mosquito Repellent Activity of *Bixa orellana* from Brazilian Amazon Region. Journal of Chemistry 2013; 1-10.

14.Castello MC , Phatak A, Chandra N and Saron M, Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixaorellana* L. Indian J Exp. Biol 2002;40:1378-1381.

15.CK Kokate; AP Purohit; SB Gokhale. Pharmacognosy, Nirali Prakashan, Pune, 2007; pp.122-135.

16. JB Harbone . Phytochemical methods, London:Chapman and Hall, Ltd. 1973, pp.49-188.

17. GE Trease; WC Evans; Trease and Evans. Pharmacognosy, A Physician's Guide to Herbal Medicine, 13th ed., Bailliere Tindall, London**,** 1989, pp.912.

18. 423, OECD, Guideline for the Testing of Chemicals, Guidance document on acute toxic class method, 2001.

19. SC. Pal and A. Nandy, Antiinflammatory, analgesic and antipyretic activity of *Achras Sapota Linn*. Leaf extracts and its isolated compounds, *Indian Drugs*, 1999,36,106.

20. O .Arthur, DC. Grafton, R. Alfonso et al. *Remington's Pharmaceutical Sciences*. Mack Publishing Company, Easton, Pennsylvania, 1980, 1461.

21.Shoba FG, Thomas M**:** Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. *J Ethnopharmacol* 2001, 76(1):73-76

22. S.Bolton, Analysis of variance. In: Pharmaceutical statistics-practical and clinical application. Marcel Dekker NY 1997,2(1).