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Phytochemical investigation and Evaluation of Anticonvulsant activity *Bixa orellana Linn.* Bark

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ABSTRACT: Aim: The present investigation was aimed to investigate the phytochemical constituents and anticonvulsant activity of ethanolic bark extracts of *Bixa orellana Linn.* **Method:** The phytochemical investigation was done by using various chemical analyses and standard methods. The extracts were investigated for acute toxicity and LD_{50} was calculated. The anticonvulsant effect of ethanolic extract was studied by using maximum electroshock (MES) and pentylenetetrazole (PTZ) in male mice. The bark extract of *B. orellana* (orally) was administered in mice at the doses of 100 and 200 mg/kg. **Result:** The extract suppressed hind limb tonic extensions (HLTE) induced by Maximal electroshock (MES) and also exhibited protector effect in PTZ-induced seizures, at 200 mg/kg dose. **Discussion:** Data from this study shows that the extract significantly increases the onset time and decreases the duration of seizures by electroconvulsive shock. The study also revealed that the onset of tonic convulsion produced by PTZ was significantly delayed and also duration of seizures was prolonged. **Conclusion:** It is concluded that the ehanolic bark extract of *B. orellana* exerted the significant anticonvulsant effect.

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INTRODUCTIONS:

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons [1]. Globally, there are nearly 50 million people suffering from epilepsy, 80 % of which are in the developing countries and 90 % of these do not receive appropriate treatment. India alone has approximately 8-10 million epileptics. Epilepsy affects not only the individual, but also has consequences for the family and the rest of society [2]. 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. *Bixa orellana* Linn. (*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]. *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 × 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 × 1-2 cm, pinkish, whitish. Fruit a spherical or broadly elongated ovoid capsule, 2-4 × 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 coloring agent, it is also used to color 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].

MATERIAL AND METHODS:

Collection and authentification of plant:

The barks of Bixa orellana were collected from the Herbal garden of Jeypore College of Pharmacy, Jeypore, Koraput district, Odisha, India, in the month of November 2017. 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-407/17, dated 9.12.2017. After authentification bark were collected in bulk and washed under running tap water to remove adhering dirt. Then the barks were shade dried. The dried materials were made into coarse powder and stored in a closed air tight container for further use.

Drugs and Chemicals:

Diazepam was procured from Ranbaxy, India, and Pentylenetetrazole from Sigma, USA as a gift sample. The ethanol AR procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. All other chemicals and reagents used in present work were procured from authorized dealer.

Preparation of extracts:

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol as solvent. A total amount of 650 g coarse powder was extracted with 800 ml of solvent. About 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain the crude extract. The crude extracts were kept in closed air tight containers under cool and dark place for further study [13,14].

Priliminary phytochemical investigation:

The crude ethanol extracts of the bark 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 [14,15].

Animals:

Albino mice of either sex weighing between 20-30 g were used from the experiment from the animal house of Jeypore College of Pharmacy, Jeypore, Odisha. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet. Water was allowed *ad libitum* under hygienic conditions at room temperature with 12 h light and dark cycles. All the studies conducted were approved by the Institutional Animal Ethical Committee (1906/PO/Re/S/16/CPCSEA), Jeypore College of Pharmacy, Jeypore, Odisha, 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 study:

The acute toxicity of bark extracts of *B. orellana* was determined by using albino mice of either sex weight between (20-25 g), maintained under standard conditions. The animals were fasted for 3 h prior to the experiments. Animals were administered with single

dose of ethanol bark extract of *B. orellana* and observed for its mortality up to 48 h study period (short term toxicity). Based on the short-term toxicity profile, the next dose was decided as per OECD guidelines No 425. Since no mortality was observed up to dose 2000 mg/kg from the LD50 dose, 100 mg/kg and 300 mg/kg doses were selected and considered as low and high doses respectively [17].

Table 1. Effect of ethanolic bark extract of *B. orellana* on tonic seizures induced by maximal electroshock in mice.

Treatment Group	Dose mg/kg (PO)	Onset time (S)	Duration of HLTE (S)	% IOC
Control (Gr. I)	1ml/kg	2.16±0.38	105.2±2.73	-----
Diazepam (Gr. II)	4	0	0	100
Ethanolic extract (Gr. III)	100	5.43±0.67*	61.14±1.8*	47.1
Ethanolic extract (Gr. IV)	200	12.04±0.73*	33.57±4.1*	68.7

Values are given as mean ± SEM, for six mice in each group. Results are statistically significant at *P<0.001 as compared with control. IOC - Percentage inhibition of convulsions.

Experimental Methods (MES):

In maximum electroshock induced seizure model, Electroconvulsive shock (50 mA for 0.2 s) was delivered through ear electrodes to induce hind limb tonic extensions (HLTE) in mice. The extract was administered orally at the doses of 100 and 200 mg/kg into test groups. Gum acacia in water and Diazepam (4 mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. Electroconvulsive shock was delivered 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted closely for 2 min. The animals that did not exhibit HLTE were considered protected. Percentage of inhibition of seizures relative to controls was calculated and in PTZ Induced seizures method, PTZ at the dose of 80 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic tonic convulsions in mice. Doses of 100 and 200 mg/kg of the extract were administered orally into test groups. Gum acacia in water and Diazepam (4 mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected. Percentage of inhibition of seizures relative to controls was calculated [18-22].

STATISTICAL ANALYSIS:

The data are represented as mean ± standard error of mean (SEM), and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey post test where $P < 0.001$) was considered statistically significant [23,24].

RESULTS AND DISCUSSION:

The preliminary phytochemical screening showed that the ethanolic extracts of *B. orellana* bark contains Alkaloids, Flavonoids, Terpenoids, Saponins, Glycoside, Steroids and Tannins and Carbohydrates absent in the extracts**.** In maximum electroshock induced seizure model, Albino mice pretreated with the ethanolic extract have been significantly protected from convulsions induced by electroshock one hour post-dosing. The percentage inhibition achieved at the doses 100 and 200 mg/kg were 47% ($p<0.001$) and 69 % ($p<0.001$) respectively. Extract at both the doses, prolonged the onset of convulsions in the extract treated group compared to vehicle treated control group (Table 1).

Table 2. Effect of ethanolic bark extract of *B. orellana* on Pentylenetetrazole induced Seizures in mice.

Treatment Group	Dose mg/kg (P.O)	Onset time (S)	Duration of HLTE (S)	% IOC
Control (Group-I)	1ml/kg	56.6±2.28	46.17±3.11	-----
Diazepam (Group-II)	4	0	0	100
Ethanolic extract (Group-III)	100	87.2±2.14*	32.26±1.67*	31.73
Ethanolic extract (Group-IV)	200	96±1.43*	18.07±1.57*	63.71

Values are given as mean + SEM, for six mice in each group. Results are statistically significant at *P<0.001 as compared with control. IOC - Percentage inhibition of convulsions.

So in PTZ methods, Animals treated with ethanolic extract at a dose of 200 mg/kg showed alteration in the occurrence of HLTE and duration of seizures significantly as related to controls in the model of convulsion induced by pentylenetetrazole in mice but did not alter at 100 mg/kg. Percentage of inhibition of seizures for 200 mg/kg relative to controls was 43.18 % (Table 2).

CONCLUSION:

Based on the above investigations, it may be concluded that the ethanolic extract of bark of *B. orellana* exhibited significant anticonvulsant activity. These findings justify the traditional use of root of this plant in the control and/or treatment of convulsions and epilepsy. The presence of flavanoids may partially contribute the significant activity. Further detailed phytochemical investigations are required to identify the phytoconstituents responsible for the anticonvulsant activity.

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