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Research article

Phytochemical investigation and anthelmintic activity of various leaf extracts of *Bixa orellana Linn*.

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Abstract

The present investigation was aimed at investing the anthelmintic activity of *Bixa orellena Linn*. leaves. The dried leaf powder was subjected to successive extraction with ethanol, ethyl acetate, methanol and petroleum ether extract using Indian earthworms Pheretima posthuma as test worm. Various concentrations of each extracts were tested which involved determination of paralysis time and death time of the worm. Albendazole (10mg/ml) was used as a reference standard. The result of the present study indicates that among all the extract, ethanol and methanol showed dose dependant & significant anthelmintic activity. The ethanol extract showed better activity as compared to reference drug albendazole.

Key words: *Bixa orellena;* anthelmintic activity; albendazole.

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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 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 [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].

Materials and methods

Drugs and chemicals

Albendazole was procured as gift sample from Sri Pharmacare, 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 and petroleum ether AR 40-60°C from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals reagents used in the present work were procured from authorized dealers.

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 authentication, 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 [13,14].

Preliminary phytochemical investigation

The crude ethanol, ethyl acetate, methanol and petroleum 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[14,15].

Table 1. Phytochemical screening for the different solvent extracts of *Bixa orellana leave*

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

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

Anthelmintic activity

Worm collection and authentication

The anthelmintic activity was evaluated on adult Indian earthworm Pheretima posthuma because it resembles anatomically and physiologically with the intestinal round worm parasite of human being. Indian earthworms were obtained from vermiculture area and were identified by the V.D, College (Autonomous), Dept. of Zoology, Jeypore, Koraput, Odisha, India

Preparation of Test sample

The test samples were prepared by dissolving and suspending 2.5 grams of each extract in 25 ml of distill water to obtain a stock solution of 100 mg/ml. From this stock solution, different dilutions were prepared to get concentration range of 20, 40 and 60 mg/ml.

Anthelmintic Assay

The anthelmintic activity of leave extract of *Bixa orellana* was evaluated by using method of Ajayieoba E.O. et al on adult Indian earthworms by the reported methods with minor modification. The assay was performed on adult Indian earthworm ***Pheretima posthuma*** its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings and also easy availability earthworms have been used widely for the initial evaluation of anthelmintic activity. The in-vitro anthelmintic activity was determined by releasing into 10 ml of desired formulation containing three different concentration, each of crude extract that is petroleum ether extract, ethyl acetate, n-butanol and ethanol extract (20,40 and 60 mg/ml in distilled water) were prepared. Albendazole (10 mg/ml) was used as reference standard while distilled water used as control and six worms(same type)were placed in it. Observations were made for the time taken to paralysis and/or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline water. Death was concluded when the worms lose their motility followed with fading away of their body color or not moved when dipped in warm water at 50ºc [16,17].

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.

Table 2. Anthelmintic Activity of *Bixa orellana* leave extracts

Treatment vehicle	Concentration used	Time taken (in min.)	
		For paralysis	For death
Pet-ether extract	20 mg/ml	36.12 ± 0.31	88.23±0.31
	40 mg/ml	31.15 ± 0.33	77.17±0.14
	60 mg/ml	28.11 ± 0.27	67.23±0.33
Ethyl acetate extract	20 mg/ml	31.24±0.22	73.24±0.21
	40 mg/ml	24.17 ± 0.24	67.27±0.26
	60 mg/ml	21.33 ± 0.15	53.22±0.21
Ethanol extract	20 mg/ml	12.32 ± 0.11	23.24±0.12
	40 mg/ml	9.23±0.12	17.21±0.16
	60 mg/ml	6.27±0.17	14.23±0.17
Methanol extract	20 mg/ml	18.21± 0.11	31.28±0.21
	40 mg/ml	14.11±0.23	23.12±0.14
	60 mg/ml	8.22±0.17	19.11±0.27
Albendazole	10 mg/ml	6.24±0.24	11.21±0.11
Vehicle	----------	----------	--------

The ethanol extract yielded strongly, all the phytochemicals followed by petroleum ether, methanol and ethyl acetate. The petroleum ether extract also yielded only flavonoid, glycoside and tannin at the poor presence which were shown in (Table 1). The ethanolic and methanol extract of leaves of *B. orellena* showed significant anthelmintic activity at higher concentration (60 mg/ml). The extract showed a dose dependent activity like shortest time of paralysis and death with (60 mg/ml) concentration. The ethanol extract of *B. orellena* leaf caused paralysis in 6.27 min. and death at 14.23 min. while the methanol extract showed paralysis in 8.22 min. and death at 19.11 min. as compared to the reference drug albendazole showed the same at 6.24 min. and 11.21 min. respectively.

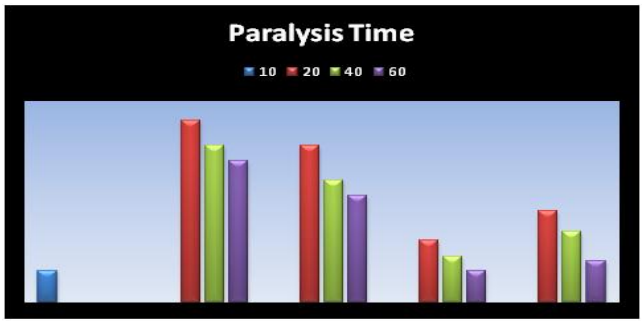

Figure 1. Anthelmintic activity of *Bixa orellana* leaves (Paralysis Time)

Figure 2. Anthelmintic activity of *Bixa orellana* leaves (Death Time)

Conclusion

Among all the extracts methanol and ethanol extract showed dose dependant & significant anthelmintic activity. Ehhanol extract showed better activity as compared to reference drug albendazole. From Phytochemical analysis, the presence of tannins as one of the chemical constituent said to possess anthelmintic activity. The folklore claim of leaves of *B. orellena* as an anthelmintic have been confirmed as the leaves extract showed activity against the earthworms used in the study. Further studies to isolate and reveal the active compound present in the crude extract of *B.orellena* leaf and to establish the MOA of anthelmintic activity.

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