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ORIGINAL RESEARCH ARTICLE

Phytochemical Investigation and Simultaneously Study on Anticonvulsant, Antidiabetic Activity of Different Leafy Extracts of *Bixa orellana* Linn

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ABSTRACT

The present study was carried out to investigate the anticonvulsant and antidiabetic activity of petroleum ether, ethyl acetate, diethyl ether, n-butanol and ethanol extracts of *Bixa orellana* Linn, leaves using experimental animal models. The extracts were screened for alkaloids, steroids, proteins, flavanoids, saponins, mucilage, carbohydrates, tannins, fats and oils. Anticonvulsant activity of above extracts was evaluated by using strychnine induced tonic convulsion of Swiss albino mice. All extracts were able to reduce convulsion in mice. The antidiabatic activity was evaluated in alloxan induced diabetic model of wistar rats. All the extracts were able to reduce sugar level in blood. Ethyl acetate and n-butanol extracts of this plant were found to have good antidiabetic activity in comparison to other extracts. The diethyl ether extracts of *Bixa orellana* was found to have good anticonvulsant activity in comparison to other extracts which was verified as statistically significant by using one way ANOVA at 1 % level of significance ($p < 0.01$).

Key words: *Bixa orellana, strychnine induced tonic convulsion, alloxan induced diabetic model*

INTRODUCTION

Bixa orellana (Bixaceae) is a tree reaching 4 to 6 meters. Leaves are entire, ovate, 8 to 20 cm long, 5 to 12 cm wide, with a broad and heart-shaped base, and a pointed tip. The flowers are white or pinkish, 4 to 6 cm diameter, 4 to 6 cm in diameter on terminal panicles. Capsules are ovoid or rounded, reddish brown, about 4 cm long and covered with long, slender and soft spines containing many small seeds covered with a dye-yielding red pulp [1,2,3].

The literature survey reveals that in traditional systems of medicine, different parts (leaves, seeds) of *Bixa*, a small tree seen throughout India, have been recommended for many more presumed beneficial uses and for the treatment of small burns, poisoning, thinning hair, headaches, gonorrhea, skin diseases, nausea and vomiting, snake bites etc[4,5].

Bixa has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardio protective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions.

MATERIALS AND METHODS

Materials

Sonicator, heating mentle, soxhlet extractor, insulin syringe, mice feeding needle, strychnine, gum acacia, stop watch, digital glucometer (Jonan and Jonsib), glucose strip, standard drug glibenclamide and Clonazepam were supplied by the department of pharmacognosy, Jeypore College of Pharmacy, Rondapalli, Jeypore. All other chemicals and reagents were procured from authorized suppliers and were of analytical grades. The leaves of *Bixa orellana* were collected from the local area Jeypore, Koraput, Odisha and authenticated by Botanical Survey of India, Shibpur, Howrah CHN/I-I/86/2011/Tech.II/254 dated 09.03.2011.

Preparation of extract of *Bixa orellana*

The whole plant was taken and air-dried in shade for ten days. Then the dried plant material was made into coarse powder. The extraction was carried out by the Soxhlet extraction apparatus using 90 % ethanol as solvent. The ethanolic extract was fractionated by extracting with the solvents such as ethyl acetate, petroleum ether, diethyl ether and n-butanol in a separating funnel.

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Then the extracts were concentrated on water bath.

Phytochemical investigation

Chemical tests were carried out on all the extracts for the qualitative determination of phytochemical constituents [6, 7].

Anticonvulsant activity

Animals

Healthy Swiss albino mice of either sex were used in the present study. They were housed in standard conditions of temperature (25±2 °C), relative humidity of 45-55 % in animal house of Jeypore College of Pharmacy. They were fed with a standard pellet diet and water *ad libitum*. Animals were caged and all operations on animals were done under aseptic condition.

Drugs

The extract of *Bixa orellana* was tested in single dose in each group of experimental model (200 mg/Kg). Clonazepam was used as the standard drug in strychnine induced tonic convulsion of Swiss albino mice model in a dose of 3 mg/Kg body weight of mice[8].

Experimental protocol

Animals were selected, weighed (20-25 g) and divided in to seven groups (n=5), namely control, standard drug and six groups belonging to five different extract of *Bixa orellana*. **Experimental Method**

The convulsion inducing agent strychnine along with vehicle gum acacia was used to evaluate the acetylcholine level lowering capacity of plant extract in Swiss albino mice. The convulsion behavior of Swiss albino mice was initiated by administration of strychnine [9].

Swiss albino mice were divided into eight groups (5 each). The first group (I) served as normal control which received distilled water only. The second group (II) served as standard control which received Clonazepam (3 mg/Kg b.w, i. p.). Groups (IV) to (VII) received a single dose of extracts of ethanol, petroleum ether, diethyl ether, ethyl acetate and n-butanol (200 mg/Kg b.w, p. o.). After 30 minutes, all animals were injected with a single dose (2 mg/Kg b.w, i. p.) of strychnine. Then onset of anticonvulsant action, number of convulsion and time of death, if any were observed for a period of one hour.

Antidiabetic activity

Animals

Healthy Wister rats of either sex were used in the present study. They were housed in standard conditions of temperature (25±2 °C) with 12 hours light per day cycle and relative humidity of 45-55 % in animal house of Jeypore College of Pharmacy. They were kept in fasting condition for 16 hours and prior to experiment they were fed with excess water at lebidum. Animals were caged and all operations on animals were done in aseptic condition.

Extract and Drugs

The extracts of *Bixa orellana* were tested in single doses in each group of experimental model (200 mg/Kg). Glibenclamide was used as the standard drug in alloxan induced diabetic model in a dose of 0.5 mg/Kg of body weight of rat.

Experimental protocol

Animals were selected, weighed (150-180 g) and devided into eight groups (n=6), namely control, diabetic control, standard drug and five groups belonging to five different extracts of *Bixa orellana*.

Experimental Method

The alloxan induced diabetic model was used to evaluate the blood sugar level reducing capacity of various extracts. Here the blood sugar level of rats was raised by administration of alloxan[10].

Wister rats were devided in to eight groups of six animals in each group. The animals were fasted for 16 hours with water adlibitum. The group-1 was served as solvent control which received the distilled water through oral route, the group-2 was served as diabetic control which received distilled water and alloxan, group-3 was served as standard control which received glibenclamide in a dose of 0.5 mg/Kg, group-4 was served as ethanolic extract in a dose of 400 mg/Kg and group-5 to 8 were received in a dose of 200 mg/Kg each in the extracts of petroleum ether, diethyl ether, ethyl acetate and n-butanol.

In sub-acute model, the administration of the standard drug was continued for seventh days once in a day. Blood samples were collected from the tip of the tail just after administration and on first, third and seven days also. The glucose level for all the samples were determined by glucometer which is previously validated for correctness.

Statistical analysis

The data on biological studies were reported as mean ± Standard deviation (n = 5). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. P values < 0.01 were considered significant [11].

RESULTS AND DISCUSSION

Phytochemical studies

(**Table 1**) shows the phytochemicals detected in *Bixa orellana* leafy extracts. The phytochemical

studies indicated that the tests for alkaloids, tannins, triterpenoids, saponin, flavonoids and sterols compounds were positive in all extract, while the tests for glycoside, gums mucilage, proteins and amino acids were negative in all extracts.

Anticonvulsant activity

The extracts of *Bixa orellana* produced a significant anticonvulsant activity after 30 minutes in the dose of 200 mg/Kg body weight as shown in (**Table 2**). The anticonvulsant activity of all the extract were found in the order of diethyl ether > ethyl acetate > petroleum ether > n-butanol.

The anticonvulsant effect of the extract obtained from different solvents was comparable with the standard drug and control used in this study. It will be worth mentioning that although different constituents were extracted in different solvents as per polarity but diethyl ether fraction is more effective as compared to other solvent extracts as, the onset of convulsion action of strychnine was delayed in case of diethyl ether fraction with minimum convulsion number, having no quantal death showing 100% survival. The activity shown by diethyl ether extract is of considerable importance and has justified its use in controlling the convulsion as suggested in the folklore medicine. By employing one-way ANOVA, all data were found to be statistically significant at 1 % level of significant ($p<0.01$) followed by Dunnett's t-test. The extent of activity shown by the crude extracts is more than that of the standard drug Clonazepam and many fold than the control, which justifies its activity as shown in (**Fig 1**).

Antidiabetic activity

The extract produced a significant antidiabetic after first, third and seventh days in the dose of 200 mg/Kg body weight, data are given in (**Table 3**). These effects were comparable with the standard drug used in the present study. It will be worth to mention that although different constituents were extracted in different solvents as per polarity but ethyl acetate and n-butanol fraction are more effective as compared to other solvent extracts. The activity showed by this extract is of considerable importance and justified its use in the diabetic control as suggested in the folklore medicines. By employing one-way ANOVA, all data were found to statistical significant at 1 % level of significance ($p<0.01$).

Antidiabetic effect against hereditary, immunological, age stress may be elicited through insulin receptors in relevant phenomena. But the extent of activity shown by the crude extracts are less than that of the standard drug, glibenclamide but many fold more than that of the control group, which justifies its activity. This can be clearly analyzed by the graphical representation shown in (**Fig 2**).

Table 1: Phytochemical screening of *Bixa orellana*

Phytochemicals	*B. orellana* Ethanolic extract	*B. orellana* Petroleum ether extract	*B. orellana* Diethyl ether extract	*B. orellana* Ethyl acetate extract	*B. orellana* n-butanol extract
Alkaloids	+	+	+	+	+
Glycoside	-	-	-	-	-
Anthraquinone glycosides	-	-	-	-	-
Gums mucilage	-	-	-	-	-
Proteins,	-	-	-	-	-
Amino acids	-	-	-	-	-
Tannins	+	+	+	+	+
Phenolic compound	-	-	-	-	-
Triterpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Sterols	+	+	+	+	+
Saponins	+	+	+	+	+
Flavones	+	+	+	+	+
Flavonoids	+	+	+	+	+
Thiol group	-	-	-	-	-

+ = Present ; - = Absent

Table 2: Anticonvulsant activity of leafy extracts of *Bixa orellana*

Group	Treatment Dose (mg/Kg)		Onset of action (Min.) (X ±	Convulsion No.(X ± SEM)	Quantal death	Survival (%)	Time of death(X ±
I	Control (Distilled water)		5.8±1.2	8.8±1.8	5	0	7.6±2.1
II	Standard (Clonazepam)	3	29.8±4.6	2.8±0.6	0	100	-
III	Ethanolic extract	200	7.4±0.9	5.8±1.8	4	20	13.2±2.7
IV	Petroleum ether	200	29.0±3.7	1.8±0.4	1	80	41.0±3.2
V	Diethyl ether extract	200	40.6±3.0	1.6±0.2	0	100	-
VI	Ethyl acetate extract	200	37.6±6.6	1.6±0.4	0	100	-
VII	n-butanol extract	200	31.4±3.1	1.8±0.5	0	100	-

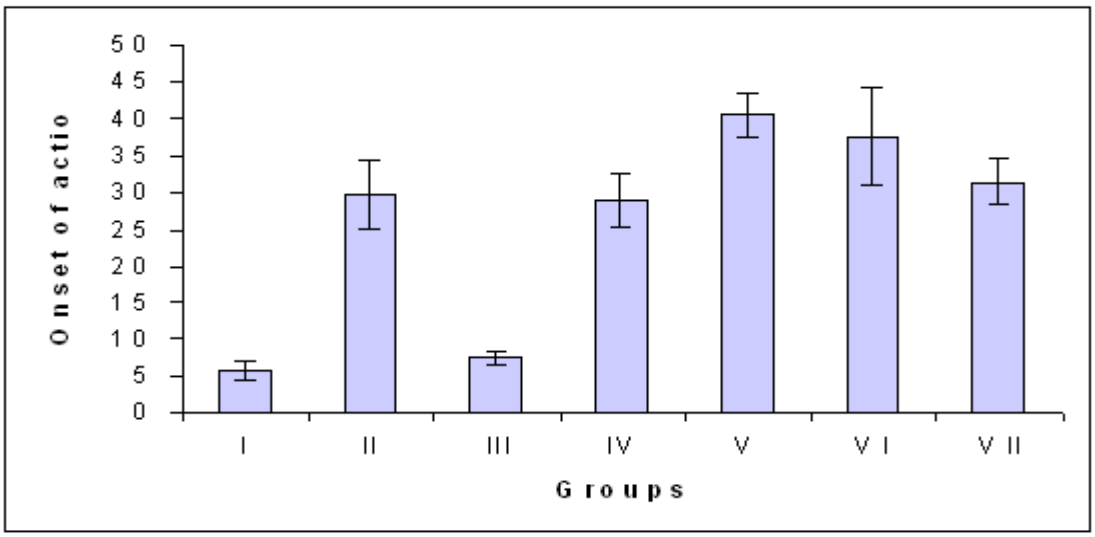
All values are expressed in mean ± standard error mean (n=5)

ANOVA DATA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1769.6257	1	1769.626	3.679251	0.001222	4.747225
Within Groups	1201.1543	12	100.0962			
Total	2970.78	13				

All values are found to be significant by using one way ANOVA at 1 % level of significance ($P < 0.01$) followed by Dunnett's T-test.

Fig 1. Onset of action of strychnine in presence of control (I), standard drug (II) and various solvent fractions.



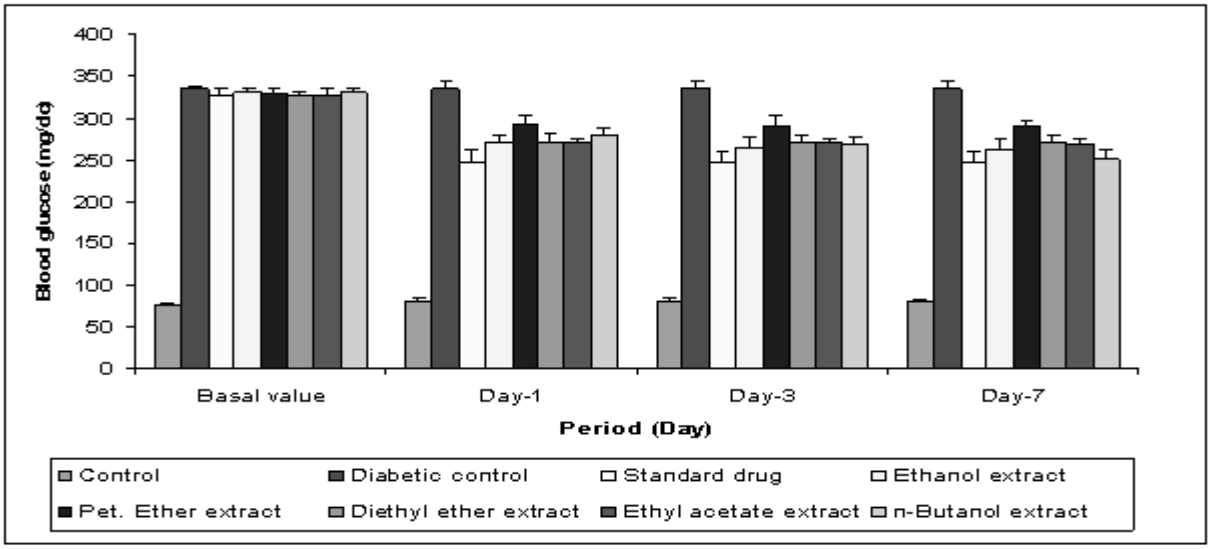
Each bar represents mean ± standard error mean.
Group III – Ethanol extract, group IV – Petroleum ether extract, group V – Diethyl ether extract, group VI – Ethyl acetate extract and group VII – n-butanol extract.

Table 3: Antidiabetic activity of total extracts of *Bixa orellana* by alloxan induced diabetic model.

Group	Treatment Dose (mg/Kg)		Blood glucose (mg/dc) (X ± SEM)			
			Basal value	Day -1	Day-3	Day-7
I	Control (Distilled water)		76.16±1.99	81.16±2.23	81.17±2.15	80.8±1.9
II	Diabetic control (Alloxan + Distilled water)	120	334.83±4.44	334.50±9.73	336.67±8.06	334.5±10.8
III	Standard (Glibenclamide + Alloxan)	0.5	327.50±6.88	247.33±15.5***	247.17±13.3***	248.3±12.2***
IV	Ethanolic extract	400	332.16±4.28	270.83±8.15***	265.33±12.2***	263.5±11.3***
V	Petroleum ether extract	200	330.00±6.27	291.50±10.7**	290.83±13.6**	290.0±8.1**
VI	Diethyl ether extract	200	327.33±4.91	272.17±9.64***	272.33±8.7***	269.3±8.7***
VII	Ethyl acetate extract	200	328.83±5.69	269.83±4.62***	269.5±5.7***	268.5±7.3***
VIII	n-butanol extract	200	330.83±6.27	279.33±8.71***	268.83±7.4***	250.7±10.4***

All values are expressed in mean ± standard error mean (n=6), **p<0.01, ***p<0.01 Vs. Group-II.

Fig. 2: Comparative antidiabetic activity of different extracts of *Bixa orellana* in one concentration (200 mg/Kg) except ethanol extracts (400 mg/Kg).



Each point represents mean ± standard error mean (n=6).

CONCLUSION

It could be concluded that the leaves of plant *Bixa orellana* is having anticonvulsant, antidiabetic activities as suggested in the folklore medicines. It is also observed that the plant *Bixa orellana* is having anticonvulsant activity and better result is obtaining from extract of diethyl ether and having antidiabetic activity and better activity are recorded from the extracts of ethyl acetate and n-butanol. However, further study is required to identify the chemical constituents (At molecular level) present in extracts of this herb and responsible for the biological activity.

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