



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203





Human Journals

Research Article

January 2018 Vol.:11, Issue:2

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Evaluation of Anti – Anxiety Activity of Ethanolic Extract of the Leaves of *Costus pictus* D. Don in Swiss Albino Mice

	
Philadelphia B.V. Denzil Colaco*¹, Imtiyaz Ansari², Dr. Vanita Kanase³, Dr. Sudha Rathod⁴	
<i>*I.M. Pharm student, Department of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi Mumbai- 400705, Maharashtra, India.</i>	
<i>2. Assistant Professor, Department of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi Mumbai- 400705, Maharashtra, India.</i>	
<i>3. Head of Department, Pharmacology, Oriental College of Pharmacy, Sanpada, Navi Mumbai 400705, Maharashtra, India.</i>	
<i>4. Principal, Oriental college of Pharmacy, Sanpada, Navi Mumbai-400705, India.</i>	
Submission: 30 December 2017	
Accepted: 5 January 2018	
Published: 30 January 2018	

Keywords: Anti-anxiety activity, *Costus pictus* D. Don, Elevated Plus Maze Model, Light - Dark Box test Model.

ABSTRACT

Anxiety is a feeling of unease, such as worry or fear that can be mild or severe. Anxiety disorders are associated with distress, morbidity and mortality. The anxiety disorders include panic disorder (PD), generalized anxiety disorder (GAD), social anxiety disorder (SAD), and post-traumatic stress disorder (PTSD). *Costus pictus* D. Don formerly known as insulin plant is a species of herbaceous plant in Costaceae family native to Mexico, traditionally known in India due to its Anti-diabetic property. Further, various phytochemical investigations reveal the presence of Flavanoid (quercetin) and hydrocinnamic acid derivative (sinapic acid) which is known to reduce anxiety. This work was an attempt to explore Anti-anxiety activity of an Ethanolic extract of the leaves of *Costus pictus* D. Don in Swiss albino mice in comparison with diazepam. The ethanol extract was given in Swiss Albino Mice at a dose of 100 mg/kg body weight and 200 mg/kg body weight. Anti-anxiety activity was assessed by using Elevated Plus Maze and Light - Dark Box test Model. The results are promising for further investigation of efficient Anti-anxiety activity.



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INTRODUCTION

Anxiety is a feeling of uneasiness, such as worry or fear that can be mild or intense. It refers to a collection of mental syndromes characterized by abnormally high levels of distress and avoidance. Anxiety disorders are manifested by hyperarousal of the central nervous system and intense feelings of fear, worry or apprehension. These disorders are highly prevalent and associated with substantial distress, morbidity and mortality. Anxiety expressed as physical, emotional, and behavioural responses to perceived threats, is a normal part of everyday life. Although, Anxiety disorders form the most common type of psychiatric disorders, yet fewer than 30% of persons with anxiety disorders seek treatment. The side effects of anti-anxiety agents are feelings of insecurity, irritability/restlessness as reflected by worries/trembling/crying easily tendencies, Fears / Phobia (Fear of the dark, fear of strangers, fear of being alone, fear of animal).^[1]

Costus pictus D. Don formerly known as insulin plant or Painted Spiral Ginger. It is a species of herbaceous plant in **Costaceae** family native to Mexico, traditionally known in India due to its Anti-diabetic property. It has nice narrow long leaves with characteristic wavy edges. It can be recognized by its yellow flowers with red spots and stripes, stem with spiral leaves and light airy and tissue paper like flowers. Red painted stem enhances the beauty of *Costus pictus D. Don*. Phytochemical investigations reveal the presence of chemical constituents such as carbohydrates, triterpenoids, proteins, alkaloid, tannins, saponins, flavonoids, steroid, and appreciable amounts of trace elements.^[2]

MATERIALS AND METHODS

Plant Material -

Fresh *C. pictus* leaves were collected from the Medicinal garden of Oriental College of Pharmacy, Navi – Mumbai. The fresh plant specimen was identified and authenticated at Blatter Herbarium, St. Xavier's College, Mumbai. Leaves were washed, shade dried and powdered.

Preparation of Extract

The powdered samples were extracted with ethanol by soxhlet apparatus for a defined period with continuous agitation. The extracts were then filtered, condensed and stored for further studies. [3] [4]

Preliminary phytochemical screening

The preliminary phytochemical test of the ethanolic extract of dried leaves of *Costus pictus D. Don* was performed for the presence of various active principles (tannins, saponins, flavonoids, quinines, glycosides, cardiac glycosides, triterpenoids, phenol, alkaloids, steroids, oils and fats, phytosterols) using standard procedures. [3] [4]

Animal

Animals required for research (studying acute toxicity and anti-anxiety activity respectively) were approved from IAEC of Oriental College of Pharmacy, Sanpada, Navi Mumbai 400 705. IAEC Proposal Number: OCP/IAEC/2016-2017/02. The animals used for the study were procured from Bombay Veterinary College, Parel, Mumbai 400 012. Animals procured were specifically female Swiss Albino mice weighing 20-25 gm. The Animals were allowed to acclimatize for a period of 7 days, housed under standard conditions of temperature ($25\pm 2^{\circ}\text{C}$) and relative humidity (30%–70%) with a 12:12 light-dark cycle, fed with pellet diet and water.

Drugs and Chemicals

The Diazepam as free sample which was the leftover stocks of the college that were used as the standard anti-anxiety drug. Distilled water was used as vehicle.

Acute Toxicity Study

The acute toxicity of ethanolic extract of *Costus pictus D. Don* was determined in female Swiss albino mice. Animal was fasted overnight prior to the experiment.

The method followed to perform the AOT was the Acute Toxic Class method i.e. OECD – 423.

1. Three doses were chosen from the Annex II of OECD 423 i.e. Minimum, Medium and Maximum i.e. 50 mg, 300 mg and 2000mg after sighting study.
2. Total of 9 animals were chosen i.e. 3 animals per group and three groups were taken namely) *Costus pictus D. Don* Extract - 50mg, 300mg, 2000 mg.
3. A single dose was administered and animals were observed for a period of 14 days for clinical signs and mortality. ^[5]

Selection of Dose for Pharmacological Screening.

The ethanolic extract was found to be nontoxic up to a dose of 2000 mg/kg and did not cause death, therefore, it was considered to be safe. Hence, one-tenth of this dose, that is, 200 mg/kg body weight and half of the one-tenth dose, that is, 100 mg/kg, were used for the elucidation of anti-anxiety activity. The extracts were suspended in the vehicle in such concentrations so as to administer 100 and 200 mg/kg doses to mice through oral route. All drugs were freshly prepared before each experiment. DPZ (5mg/kg; p.o) used as standard.

Experimental Design

The animals were divided into four groups of six mice each for each activity. The drugs were administered as shown below:

1. Group I - Normal control (0.5ml of Distilled water).
2. Group II - standard (0.5ml of diazepam 5mg/kg).
3. Group III - 0.5ml of 100mg/kg extract of *Costus pictus D. Don*.
4. Group IV - 0.5ml of 200mg/kg extract of *Costus pictus D. Don*.

SCREENING OF ANTI-ANXIETY ACTIVITY:

MODEL 1- - ELEVATED PLUS MAZE

Procedure-

The Elevated Plus Maze apparatus consisted of four arms elevated 30 cm above the floor, with each arm positioned at 90° relative to the adjacent arms. Two of the arms enclosed with high walls (30 × 7 × 20 cm), and the other arms connected via a central area (7 × 7 cm) to

form a plus sign. The maze floor and the walls of enclosed arms were painted black. The room was illuminated with a 40-W lamp at the central platform. The animals were treated with vehicle, extract and diazepam orally, 60 min prior to the test. Each mouse was individually placed on the central platform facing toward an open arm. The frequency and duration of entries into the open and closed arms were observed for 5 min. An entry was counted when all four paws of the mouse entered an open or closed arm. Subsequently, the percentage of time spent (duration) in the open arms [$100 \times \text{open} / (\text{open} + \text{enclosed})$] and percentage of the number of open arm entries (frequency, $100 \times \text{open} / \text{total entries}$) were calculated for each animal. The apparatus was thoroughly cleaned after each trial. ^{[6][7][8][9]}

MODEL 2 – LIGHT DARK BOX TEST

Procedure –

The Light and Dark box Test apparatus consisted of open top wooden box. Two distinct chambers, a black chamber (25 cm long \times 35 cm wide \times 35 cm deep), painted black and was made dark by covering its top with black plywood, and a bright chamber (25 cm long \times 35 cm wide \times 35 cm deep), painted white and brightly illuminated with 40-W white light source, was placed 25 cm above the open box. The two chambers were connected through a small open doorway, (7.5 cm long \times 5 cm wide) situated on the floor level at the center of the partition. The mice were placed individually in center of the lightbox after 60 min of oral treatments and observed for 5 min. ^{[10] [11]}

Statistical analysis

The values were expressed as (n=6) mean \pm SEM. The results were subjected to statistical analysis by using two way analysis of variance (ANOVA) followed by Turkey's multiple comparisons tests using Graph pad Prism 7.

RESULTS AND DISCUSSION

Table 1- Preliminary Phytochemical screening of Ethanolic extract of leaves of *Costus pictus D. Don.*

Phytochemicals	Ethanolic Extract
Tannins	+ve
Saponins	+ve
Flavonoids	+++ve
Quinones	+ve
Glycosides	+ve
Cardiac glycosides	+ve
Triterpenoids	+ve
Phenol	+ve
Alkaloids	+++ve
Steroids	+ve
Oil and fats	+ve
Phytosterols	++ve

Acute Toxicity Study

During acute toxicity studies, the extract produced no adverse effects at dose 2000 mg/kg and did not cause any death up to a dose of 2000 mg/kg in mice. ^[5]

ANTI-ANXIETY ACTIVITY

MODEL 1- ELEVATED PLUS MAZE

Table 2: Readings of Anti-anxiety activity by using Elevated plus maze test in mice

Groups	Dose	No of Entries (sec)		Time spent (sec)			
		Open arm	closed arm	Open arm	closed arm	% OAE	% TSOA
Normal	1 ml Distilled water	1.167± 0.167	9.833± 0.543	51.667± 6.146	248.333± 6.146	10.60 %	17.22 %
Standard	5mg/kg Diazepam	15.000± 0.577	0.000± 0.000	300.000± 0.000	0.000± 0.000	100 %	100 %
Extract Test -1	100 mg Low Dose	7.333± 0.667****	5.167± 0.872****	170.667± 6.883****	129.333± 6.883****	58.66 %	56.88 %
Extract Test -2	200 mg High Dose	9.333± 0.422****	3.333± 0.422****	223.833± 4.888****	76.167± 4.888****	73.68 %	74.61 %

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by **two way** analysis of variance(ANOVA) followed by Turkeys Multiple Comparisons Test.

****P<0.0001 Vs standard

MODEL 2 - LIGHT DARK BOX TEST

Table 3: Readings of Anti-anxiety activity by using Light Dark box Test in mice

Group	Dose	Time spent in light (sec)	Time spent in dark (in sec)
Normal	1 ml Distilled water	93.167 ± 8.380	206.833± 8.380
Standard	5 mg/kg Diazepam	234.667 ± 4.264	65.333 ± 4.264
Extract Test -1	100 mg Low Dose	156.167 ± 2.151****	143.833 ± 2.151****
Extract Test -2	200 mg High Dose	186.167 ± 5.468****	113.833 ± 5.468****

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by **two- way** analysis of variance (ANOVA) followed by Turkeys Multiple Comparison Test.

****P<0.0001 Vs standard

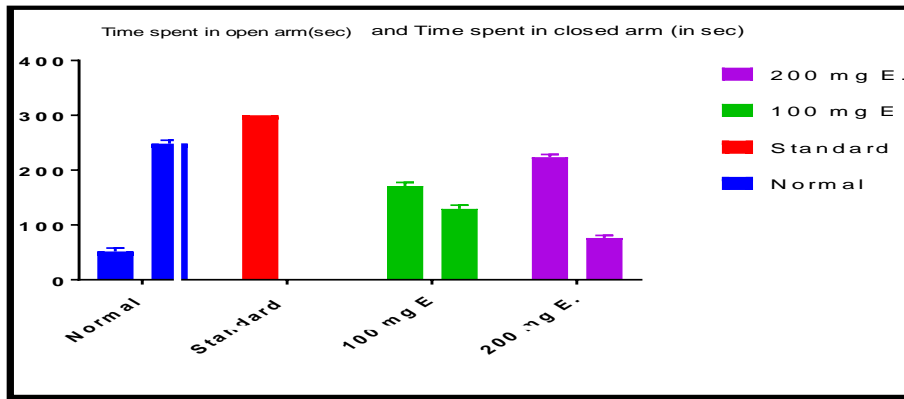


Figure 1 - Time spent in Open arm (sec) and Time spent in closed arm (sec) respectively

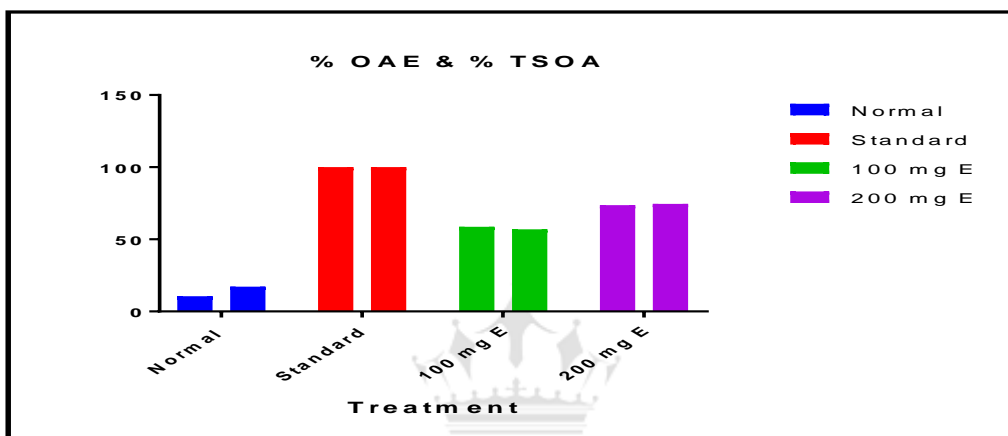


Figure 2 - % OAE & % TSOA

(% OAE – Percentage of Open Arm Entry, % TSOA - Percentage of Time spent in Open Arm respectively)

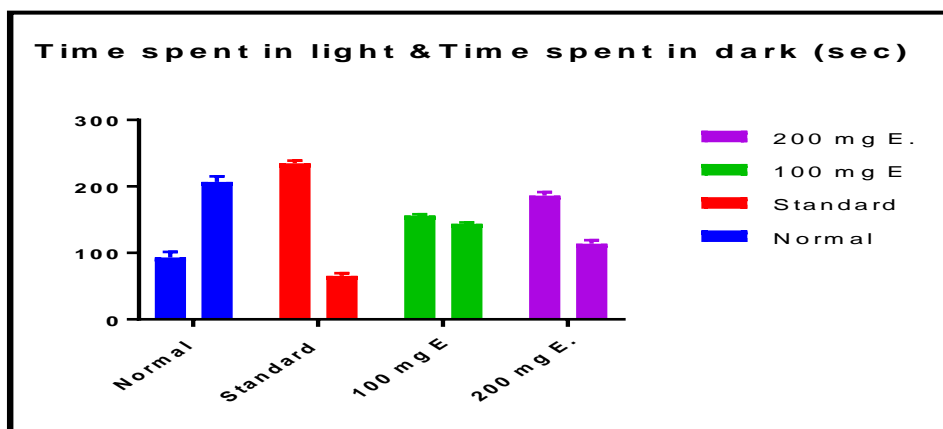


Figure 3 - Time spent in light (sec) and Time spent in dark (sec) respectively

It was observed that both the doses showed significant results. From the p values and the statistical analysis, the test – 2 (200mg/kg dose) was found to be most effective when compared to test -1. (100mg/kg dose) in both the models. Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing flavonoids (quercetin) and organic compounds like sinapic acid possess activity against many CNS disorders.^[12] Phytochemical tests of *Costus pictus D. Don* revealed the presence of flavonoids (quercetin).^{[12][13][14]} It may possible that the mechanism of anxiolytic action of *Costus pictus D. Don* could be due to the binding of any of these phytochemicals to the GABAA-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the GABAA receptor. The plant *Costus pictus D. Don* also contains hydrocinnamic acid derivative (sinapic acid) which may be responsible for its anxiolytic activity.^{[12][13][15][16]} So the anxiolytic activity of *Costus pictus D. Don* might involve an action on GABAergic transmission.^{[17][18]}

CONCLUSION

The presents study sheds light on the potential role of *Costus pictus D. Don* in ameliorating the clinical signs and symptoms by virtue of its anti-anxiety potential. The herb, besides being an effective traditional antidiabetic medicine in India may also play a major role in calming down anxiety. The study suggests that the ethanolic extract of *Costus pictus D. Don leaves* has anti-anxiety action. As the comparison is done with centrally acting benzodiazepine group of drug diazepam, it is assumed that the anti-anxiety effects of *Costus pictus D. Don* could be due to the interaction of flavonoids of the plant with the GABA/benzodiazepine receptor complex in brain. Still, extensive research is needed to synthesize new molecule with anti-anxiety activity from *Costus pictus D. Don*.^[18]

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