

PHYTOCHEMICAL AND *IN VITRO* ANTHELMINTIC STUDIES OF HYDRO-ALCOHOLIC EXTRACT OF *COSTUS PICTUS* D. DON

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ABSTRACT

Costus pictus D. Don, commonly known as spiral ginger is an ornamental plant in Indian state of Kerala. The fresh leaves of this plant is traditionally used in treatment of diabetes. The present study is to screen the phytochemical constituents of the hydro-alcoholic extract of the *Costus pictus* D. Don rhizome and to explore its anthelmintic activity. The hydro-alcoholic extract was prepared by soxhlet extraction of the dried, powdered rhizome and was subjected to preliminary phytochemical analysis. The *in-vitro* anthelmintic activity screening of the extract was performed with earthworm *Eudrilus eugeniae*. The phytochemical screening confirmed the presence of carbohydrates, phytosterols, saponin glycosides, flavanoids and tannins in detectable amount. The evaluation of anthelmintic activity against the standard drug Piperazine citrate showed significant results at same concentration (0.5, 0.75 and 1 gm%). This study provides a clear evidence for using the hydro-alcoholic extract of *Costus pictus* D. Don rhizome in the treatment of helminthiasis.

Keywords: Anthelmintic activity, *Costus pictus* D. Don, *Eudrilus eugeniae*, Hydro-alcoholic extract, Phytochemical screening.

INTRODUCTION

Natural products and their derivatives including synthetic analogs represent over 50% of all drugs in clinical use, along with those derived from higher plants representing the 25% of the total [1]. The World Health Organization (WHO) estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine includes the plant extracts. From this data, it is clear that about 4 billion people in the world rely on plant-derived medicines [2]. The WHO also publishes an astonishing report of parasitic worm infections in nearly 2 billion people in the world. In addition to this, the parasitic worms also infect livestock and crops, resulting adverse affect on food production and thereby economy. However, there are several synthetic drugs are known in the treatment of these infections, certain parasitic infections shown resistance to these drugs. Due to these reasons, the anthelmintic drug market focused on plant-derived drugs, as replacement for some of the existing synthetic drugs, which are not effective in the treatment [3].

Costus pictus D. Don, syn. *Costus mexicanus* Liebm. (Costaceae), commonly known in names such as Spiral ginger, Stepladder or Insulin plant, is indigenous to Mexico. In India, the plant is grown in gardens as ornamental plant especially in state of Kerala. The studies on extract of *Costus pictus* leaves showed antidiabetic activity [4], while the leaves and rhizome of other species of *Costus* are known for the antidiuretic [5], anthelmintic [6], antibacterial [7] and antitumor [8] activities. Present study intends to perform the preliminary phytochemical screening of hydro-alcoholic extract of dried rhizome of *Costus pictus* D. Don and to evaluate the anthelmintic activity of the extract using earthworm *Eudrilus eugeniae*.

MATERIALS AND METHODS

Plant materials

Fresh plant of *Costus pictus* D. Don was collected from the cultivated plants in the farm of Department of Agriculture, Ulloor, Thiruvananthapuram, Kerala. The plant was identified by Department of Pharmacognosy, Govt. Ayurveda College, Poojappura, Thiruvananthapuram. The voucher specimen of the plant was deposited in the College of Pharmaceutical Sciences, Govt. Medical College Thiruvananthapuram, Kerala, India.

Preparation of plant extracts

The collected rhizomes were washed, sliced, shade dried and powdered (#40 mesh). 100gm of the powdered rhizomes was

subjected to Soxhlet extraction [9] with aqueous alcohol (30/70). The extract was then evaporated to complete dryness under vacuum.

Phytochemical evaluation

Phytochemical evaluation of the hydro-alcoholic extract was carried out using standard chemical test protocols [10].

Animals

Adult earthworms *Eudrilus eugeniae* [11] used for the anthelmintic activity study were procured from the University of Agriculture, Vellayani, Kerala and washed with normal saline to remove all the adhering fecal matter. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used due to its anatomical and physiological resemblance with the human intestinal roundworm parasites [12].

Evaluation of anthelmintic activity

The evaluation of anthelmintic activity was performed by comparing the results of hydro-alcoholic extract with that of standard drug Piperazine citrate [13]. The standard, Piperazine citrate was diluted with normal saline to obtain 0.5, 0.75, 1.0 gm%, poured into petridishes. The test compound was dissolved in minimum quantity of DMSO and diluted to 15 ml with normal saline to obtain 0.5, 0.75, 1.0 gm%. Normal saline served as the control for standard. Six earth worms of nearly equal size were placed in each petridish at room temperature. The time taken for complete paralysis and death along with mean paralysis time and mean lethal time were recorded for each sample. Observations were made for the time taken for paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color [14, 15].

Statistical analysis

The results were analyzed for statistical significance using one way ANOVA followed by Tukey post hoc test [16]. Difference at $P < 0.01$ was considered as significant.

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical evaluation of secondary phytoconstituents are listed in Table 1. The phytochemical evaluation confirmed the presence of carbohydrates, steroids, saponin glycosides, flavanoids, tannins and phenolic compounds.

Table 1: Qualitative Phytochemical evaluation of hydro-alcoholic extract of *C. Pictus* rhizome

| S. No. | Tests/ reagents used | Result |
|--------|---|--------|
| 1 | Test for carbohydrates | + |
| 2 | Test for proteins | - |
| 3 | Test for Fats and Oils | - |
| 4 | Test for steroids | + |
| 5 | Test for glycosides | |
| | 1. Cardiac glycosides | - |
| | 2. Anthraquinone glycosides | - |
| | 3. Saponin glycosids | + |
| | 4. cyanogenetic glycosides | - |
| | 5. Coumarin glycosides | - |
| | 6. Flavanoids | + |
| 6 | Test for alkaloid | - |
| 7 | Test for tannins and Phenolic compounds | + |

Anthelmintic Activity

The results of anthelmintic activity studies of the hydro-alcoholic extract are summarized in Table 2 and 3. Table 2 shows the comparison of anthelmintic activity of hydro-alcoholic extract against the standard drug piperazine citrate for paralysis group. The

results obtained for the hydro-alcoholic extract are comparable with the standard drug Piperazine citrate, at same concentration. In case of paralysis, there is no significant difference in time taken for paralysis of worms in hydro-alcoholic extract and standard drug Piperazine citrate (see table 2). The activity of hydro-alcoholic extract against piperazine citrate for the death group is given Table 3.

Table 2: Comparison of anthelmintic activity of hydro-alcoholic extract against the standard drug piperazine citrate for paralysis group

| Concentration | Compound | Mean time (min.) | S. D | t | P |
|---------------|-------------------------|------------------|------|------|--------|
| 0.5 gm% | Hydro-alcoholic extract | 61.50 | 4.23 | 0.64 | p>0.05 |
| | Piperazine citrate | 59.50 | 6.32 | | |
| 0.75 gm% | Hydro-alcoholic extract | 38.17 | 3.76 | 1.08 | p>0.05 |
| | Piperazine citrate | 40.33 | 3.14 | | |
| 1.0 gm% | Hydro-alcoholic extract | 23.67 | 2.73 | 0.10 | p>0.05 |
| | Piperazine citrate | 23.50 | 2.88 | | |

Table 3: Comparison of anthelmintic activity of hydro-alcoholic extract against the standard drug piperazine citrate for death group

| Concentration | Compound | Mean Time (min.) | S.D. | t | P |
|---------------|---------------------------|------------------|------|-------|--------|
| 0.5 gm% | Hydro - alcoholic extract | 76.17 | 1.72 | 11.50 | p<0.01 |
| | Piperazine citrate | 91.83 | 2.86 | | |
| 0.75 gm% | Hydro - alcoholic extract | 49.17 | 6.71 | 6.14 | p<0.01 |
| | Piperazine citrate | 67.00 | 2.37 | | |
| 1.0 gm% | Hydro - alcoholic extract | 33.50 | 2.43 | 10.18 | p<0.01 |
| | Piperazine citrate | 51.17 | 3.49 | | |

It is noteworthy that there is a significant difference in time taken for the death of worms in hydro-alcoholic extract in comparison to Piperazine citrate (p<0.01). It is evident from the experimental data that, the crude hydro-alcoholic extract of rhizome of *Costus pictus* D. Don showed significant anthelmintic activities at 0.5, 0.75 and 1 gm%.

CONCLUSION

The preliminary phytochemical screening and anthelmintic activity studies were carried out on hydro-alcoholic extract of *Costus pictus* D. Don rhizome. The phytochemical screening showed the presence of carbohydrates, phytosterols, saponin glycosides, flavanoids and tannins in detectable amount. Anthelmintic activity study of the extract confirmed significant activity at a dose level of 0.5, 0.75 and 1 gm%. The extract showed same activity on paralysis, while significantly higher activity in death of the worms compared to that of standard drug Piperazine citrate at the same concentration. The present results provide experimental support for using hydro-alcoholic extract of *Costus pictus* D. Don in the treatment of helminthiasis.

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