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STUDIES ON THE EVALUATION OF TOTAL FALAVANOID CONTENT AND *IN VITRO* ANTIHELMINTIC POTENTIAL OF METHANOLIC LEAF EXTRACT OF INSULIN PLANT *COSTUS IGNEUS* N. E. BAR.

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ABSTRACT

Gastrointestinal helminths are common infectious agents in human and animals causing anorexia, anemia, diarrhoea and heavy production losses. The important gastro-intestinal helminth parasites belong to three different classes, nematode (roundworm), trematode and cestodes (flatworm). Plants that act as anthelmintics have historically been used to treat parasite illnesses. *Costus igneus,* sometimes referred to as Spiral flag, is a plant that was recently imported to India from South and Central America. Plants that act as anthelmintics have historically been used to treat parasite illnesses. *Costus igneus,* sometimes referred to as Spiral flag, is a plant that was recently imported to India from South and Central America. It often grows as a decorative plant in southern India, and its leaves are used as a nutritional supplement to treat diabetes mellitus. The current study's

objective was to assess *costus igneus* anthelmintic effectiveness against *Pheretima posthuma* (earthworm). **Methods:** The methods outlined were used to evaluate the anthelmintic activity on mature Indian earthworms. **Results:** Following exposure to methanolic extract at doses of 1.0, 2.0, and 3.0 mg/ml, paralysis was detected at 9.1 ± 0.43, 8.2 ± 0.20, and death at 19.2 ± 0.50, 24.0 ± 0.32, and 26.0 ± 0.42 minutes, respectively. The results indicate that the Methanolic extract of *Costus igneus* plant has anthelmintic action in terms of dose dependent approach when connected with standard anthelmintic drug Piperzine citrate (10 mg/ml). Paralysis was observed at 4.3 ± 0.29 min and death occurred after 9.4 ± 0.35 min.

Keywords: ***Costus igneus,*** **Gastrointestinal helminths, anthelmintic activity, Falavanoid Content**

1. INTRODUCTION

Elevated plants are a major source of therapeutic compounds and are utilized extensively in both conventional and contemporary global medical systems. Currently, more than 2000 plants are used to produce more than 8000 compound formulations and more than 1000 single drugs. Thanks to India's ideal agroclimatic conditions, around 9500 medicinal plants may thrive there. India has a great potential to play a major role in the medical plant sector. Plant products contain a large number of noteworthy chemicals with practical and medicinal properties Insulin plant, sometimes called Fiery Costus, Step ladder, Spiral flag, or Insulin plant, is *Costus igneus* (Nak) [syn. (*Costus pictus* (D. Don), *Costus mexicanus* (Liebm ex Petersen), or *Costus congenitus* (Rowle)]. Its leaves aid in the body's synthesis of insulin **[1]**. The demand for natural treatments for diabetes mellitus is rising. The insulin plant is a well-known traditional herb that is often used in Ayurvedic medicine. The demand for natural treatments for diabetes mellitus is rising. The insulin plant is a well-known traditional herb that is often used in Ayurvedic medicine. The demand for natural treatments for diabetes mellitus is rising. Insulin plant is a well-known traditional herb that is often used in Ayurvedic medicine. Many people grow Costus species for their aesthetic and medicinal qualities. The main source of diosgenin, an anti-diabetic compound used to treat diabetes mellitus, is the rhizome **[2]**.

Helminthiasis, also referred to as worm infestation, is a prevalent disease and a significant global public health concern. Worldwide, helminth infections impact over 2 billion people. These infections can cause a range of serious health problems, including anemia, diarrhea, and abdominal pain. They can also hinder cognitive and physical

development, particularly in underdeveloped countries **[3]**.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals

Analytical-grade chemicals were utilized throughout the study. The standard solution was made using a 500 mg piperazine citrate tablet from Taj Pharmaceutical Ltd. in Mumbai.

2.2 Plant collection and authentication

The fresh aerial parts (leaves) for the planned work of *Costus igneus* were gathered from Ananthagiri Village, Telangana, India, and subsequently verified by Dr. K. Srinivasa Reddy, Assistant Professor, Department of Botany, Govt. Degree College for Woman, Nalgonda, Telangana. A voucher specimen was kept in herbarium voucher No. 05/HB/ANRP/2023.

2.3 Preparation of Extracts

For the extraction, plant leaves that had been dried and ground into a coarse powder were employed. Using a Soxhlet equipment and methanol solvent, repeated extractions of the plant's coarse powder were performed. The leaf components of *Costus igneus* were extracted, dried, and kept in desiccators for later use after being concentrated by rotary evaporation at 40°C under decreased pressure. It was chosen based on the yield %, color, and uniformity. The finished product was used for quantitative estimation of flavanoid, phytochemical screening, and anthelmintic activity testing. The dried extracts were utilized as a vehicle for anthelmintic activity and suspended in 0.5% CMC in distilled water.

2.4 Worms Collection and Authentication

An adult Indian earthworm known as *Pheretima posthuma* was extracted from the water-soaked soil and subsequently identified and confirmed. Earthworms measuring 3-5 cm in length and 0.1-0.2 cm in width were selected for the experiment.

2.5 Study protocol

2.5.1 Quantitative estimation of total flavonoids

The following method was used to carry out a quantitative analysis of total flavonoids present in methanolic leaf extract of *Costus igneus*. In a 250 ml beaker, 2.50 g of the sample were added to precisely 50 ml of 80 % aqueous methanol, which was then capped and let to stand for 24 h at room temperature. After removing the supernatant, the residue was extracted three more times using the same amount of ethanol. The sample went through a filter made of Whatman filter paper number 42 (125 mm). Subsequently, the filtrate was put into a crucible and dried over a water bath. A desiccator was used to cool the material inside the crucible before being weighed to

ensure its constant weight. Calculated the flavonoid percentage with following formula

% Flavonoids = Flavonoids / weight Sample weight ×100

2.5.2 *In vitro* antihelmintic potential

For the purposes of the study, six groups were made in duplicate, one with water and the other with Piperzine citrate as the standard control. For this investigation, ten individual worms with comparable weights and sizes were used. Data were collected and the concentrations of each extract were set at 1.0, 2.0, and 3.0 mg/ml. The three extracts, including the reference solution, were all freshly made just before the experiment was conducted. The earthworms were placed into each petri dish, examined for paralysis, and then left to die. When there was no visible movement other than when the parasite was very agitated, an average time for paralysis (measured in minutes) was recorded. By closely viewing with both the unaided eye and a hand lens magnifier, the time for the worms' death (measured in minutes) was recorded when they lost their ability to move and their body color faded. Piperazine citrate (10 mg/ml) was used as a reference standard. This outcome was tested three times. The activity of anthelminths is analyzed. Group II received the standard therapy (piperazine citrate), Group III, IV, and V received different dosages of methanolic plant extracts for the anthelmintic action, and Group I served as the control group. Each group's mean standard deviation of six animals was used to present all the data. (shown in **Table 2, Figure 1**).

2.6 Statistical analysis

The statistical package for social science, version 20, IBM Corporation, Armonk, New York, USA, was used to analyze the data, which were displayed as mean standard deviation.

3. RESULTS

The anthelmintic assay process underwent a few minor modifications **[4]**. Infections with parasitic worms and helminthiasis can affect humans. When the parasites mature into fully formed adult worms with a characteristic tissue distribution, they first enter humans through the skin or gastrointestinal tract (GIT). The earthworms (*Pheretima posthuma*) were treated with normal saline solution as a control. They exercised their bodies well and remained active for more than eight hours. The length of time it took for each worm to either die or become paralyzed was noted. It was stated that paralysis happens when the worms do not get even in ordinary saline. The worms' loss of motility and consequent color fading indicated their impending death. Piperazine citrate's primary effect on worms is to cause a flaccid paralysis

that eventually causes the worms to eject themselves by peristalsis. The worm muscle membrane becomes hyperpolarized and less excitable due to piperazine citrate, resulting in flaccid pralalysis and muscular relaxation **[5]**. The 10 mg/ml piperazine citrate that was synthesized was placed in a different petri plate. Group I served as the control group, Group II received piperazine citrate, the standard treatment, and Groups III, IV, and V received different dosages of methanolic plant extracts for their anthelmintic properties. The mean standard deviation of six animals from each group was used to display all the results (**Table 2, Figure 1**). The data are displayed graphically as the mean S.D of six worms in each group and are tabulated.

The most important class of polyphenols for the diet of humans is called flavonoids, and it is primarily found in plants. Consequently, **Table 1** provides a summary of the total flavonoid content of a methanolic extract of the whole *Costus igneus* plant.

Table 1: Total flavonoid content

S. No.	Phytochemical	Wt. of sample (g)	Wt. of dried filtrate (g)	% yield
	Flavonoids	5	2.1	2.3

Table 2: *In vitro* anthelmintic activity of methanolic extract of *Costus igneus* leaves

S. No.	Treatment groups	Group	Conc.(mg/ml)	Paralysis time (min) (Mean±S.D.)	Death time(min) (Mean±S.D.)
1	Conrol	I	--		--
2	Standard Piperazine citrate	II	15	--	9.4 ± 0.35
3	Methanolic leaf extracts of *Costus igneus*	III	1	4.3 ± 0.29	19.2 ± 0.50
		IV	2	9.1 ± 0.43	24.0 ± 0.32
		V	3	8.2 ± 0.20	26.0 ± 0.42

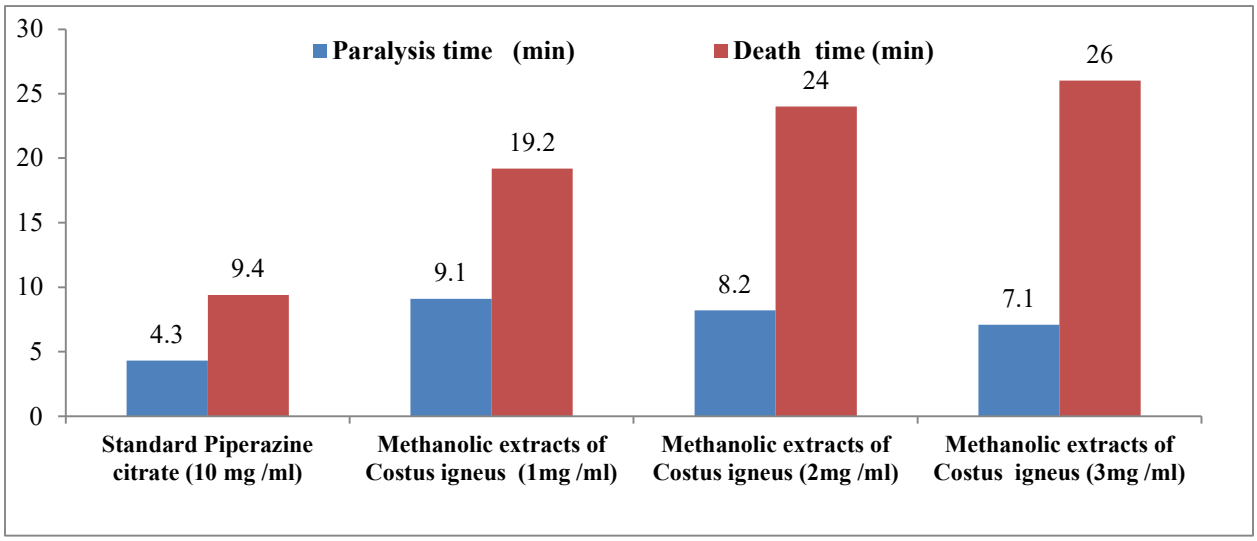

Figure 1: *In vitro* anthelmintic activity of methanolic extract of *Costus igneus* leaves

4. DISCUSSION

At 1.0, 2.0, and 3.0 mg/ml doses of the methanolic extract, paralysis was noted 9.1 ± 0.43, 8.2 ± 0.20, and death occurred 19.2 ± 0.50, 24.0 ± 0.32, and 26.0 ± 0.42 minutes after exposure, respectively. The prescribed drug, piperzine citrate (10 mg/ml), resulted in death after 9.4 minutes and paralysis after 4.3 minutes. The earthworms responded more strongly to the *Costus igneus* extracts than to the reference drug, piperzine citrate (10 mg/ml). Comparing the above results to traditional anthelmintic treatment, it is clear that the methanolic extract of the *Costus igneus* plant has dose-dependent anthelmintic action.

5. CONCLUSION

The current study showed that the methanolic extract of *Costus igneus* may have anthelmintic properties. The group of polyphenols known as flavonoids, which are typically present in plants, is the most significant one for the human diet, the pharmacological basis for the same has to be established by more research utilizing animal models. The active components that give *Costus igneus* extracts their anthelmintic properties still need to be isolated and characterized.

6. LIMITATIONS

Though there are so many accessible medicinal plants that possess anthelmintic property, there are few issues that need to be taken into account, such as whether the selected plants are complaisant to cultivation, their form of administration, their palatability, stability and biodegradability of active compounds in preserved products, their chances/probability of harmful effects/ reaction/ aftermath. Some plants have high active compound of known direct-acting parasitic ides that may be effective for short-term "curative" purpose whereas some plants should be used along with the other feed for preventive purpose **[6-10]**.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest relevant to this article.

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