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## ANTIDIABETIC AND ANTHELMINTIC ACTIVITY OF *CROSSANDRA* *INFUNDIBULIFORMIS*

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### Abstract

**Objective:** To investigate the Antidiabetic and anthelmintic activity of the ethanolic extract of the leaves of *Crossandra infundibuliformis*.

**Methods:** Antidiabetic activity was evaluated against  $\alpha$ -amylase enzyme and the anthelmintic activity was evaluated on an adult Indian earthworms, *Endrullus enguinae*.

**Results:** Antidiabetic activity was performed in five different concentrations such as 100 $\mu$ g, 500 $\mu$ g, 1000 $\mu$ g, 1500 $\mu$ g, 2000 $\mu$ g of ECI. Anthelmintic activity was performed in three different concentrations such as 50mg/ml, 100mg/ml and 150mg/ml. Both the activities were dose dependent. The highest concentration showed the maximum activity.

**Conclusions:** Our results confirm that the ethanolic extract of *Crossandra infundibuliformis* exhibited significant effect against  $\alpha$ -amylase and *Endrullus enguinae*. Antidiabetic and anthelmintic activity leads some support to the use of *Crossandra infundibuliformis* for various ailments in the traditional medicine of India.

**Keywords:** *Crossandra infundibuliformis*, Antidiabetic activity,  $\alpha$ -amylase, Anthelmintic activity, *Endrullus enguinae*, Indian earth worms.

### Introduction

*Crossandra infundibuliformis* (Acanthaceae) is an important plant in horticulture[1]. It is abundantly present in tropical areas such as South India and Sri Lanka. The flowers of *C. infundibuliformis* are sometimes referred as 'Tropical flame' or 'Firecracker' has been used as hairdo by the Puliya's tribal women. The leaf extracts of *Crossandra infundibuliformis* shows aphrodisiac, anti-inflammatory and analgesic properties. The leaf extracts also reported for wound healing, antimicrobial, antioxidant and larvicidal activities. Due to its medicinal value, this plant is used to treat various ailments. In China, some flowers have been eaten since ancient times, and some flowers have

been used in traditional Chinese medicine. Researchers reported that the fresh aqueous extract of the flowers exhibited better anti-solar activity than the dry flower extract and have concluded that the flowers of *C.infundibuliformis* can be used in various sunscreen formulations. Phytochemical screening of various solvent extracts of *C.infundibuliformis* revealed the presence of carbohydrates, flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. Diabetes is a chronic [2] metabolic disorder in which homeostasis of the carbohydrate, protein and lipid metabolism is improperly regulated by the pancreatic hormone, insulin; resulting in an increased blood glucose level i.e. hyperglycemia. The hyperglycemia associated with the incidence and progression of micro vascular (diabetic retinopathy, loss of vision and nephropathy) and macro vascular diseases (amputation and cardiovascular disease) that are difficult to manage. The prevalence of diabetes is increasing annually and the number of diabetics is projected to rise above 300 million before 2025. Parasitic infection including helminthiasis is a critical serious problem in the tropical regions including the Asian countries which affects more than two billions of people worldwide. Helminths produce serious problem in human and other animals around the world specifically to the third world countries. Different type of helminths infects the human and animals out of which intestinal round worms (*Ascardia* sp.) are most common. Approximately 300 million people suffer severe morbidity associated with these parasites and half of which are school-going children affected by massive infections. Variety of several clinical symptoms arises due to this infection include dysentery, diarrhea, nausea-vomiting, loss of appetite and weight, acidity and sometimes anaemia. Helminthiasis are also one of the most important animal diseases worldwide, inflicting heavy production losses in grazing animals, especially in developing countries, leading to mortality, chronic infections, which cause reduced productivity, fertility, growth, milk and meat production in animals. Anthelmintic drugs are used to control helminth infections, but they are associated with many side effects [3,4]. Frequent use of anthelmintic drugs leads to the development of resistance in helminthes due to several factors such as initial resistance, allele frequency, treatment frequency and refugia. Traditionally, there are two approaches that have been employed for anthelmintic studies; the first one is through offering plants or plant parts to naturally or experimentally infected animals and quantifying the consequences of their consumption.

The second approach is through testing plant extracts and decoctions derived from medicinal plants in *Invitro* and *Invivo* systems. The aim of the present study was to investigate the Antidiabetic and Anthelmintic activity of the ethanolic extract of the leaves of *Crossandra infundibuliformis* (ECI) using  $\alpha$ -amylase and Indian earth worms respectively.

## Materials and Methods

### Plant collection

The leaves of *Crossandra infundibuliformis* were collected from the South Districts of Goa in the month of October-November. The plant *Crossandra infundibuliformis* was identified and confirmed by the botanist Dr. M. K. Janarthanam, Head, Department of Botany, Goa-University, Goa.

### Extract preparation

The leaves of the *Crossandra infundibuliformis* were shade-dried and pulverized to powder in a mechanical grinder. The powder of the plant (1 kg) was extracted by maceration with solvent ethanol. The ethanol extract of the leaves of *Crossandra infundibuliformis* were used for the further studies.

### Antidiabetic activity

The inhibition assay was performed using the chromogenic DNSA method [5]. The total assay mixture composed of 1400 µl of 0.05 M sodium phosphate buffer (pH 6.9), 50 µl of amylase and samples at concentration 2000, 1500, 1000, 500 and 100 µg were incubated at 37°C for 10 min. After pre-incubation, 500 µl of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 ml DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature and the absorbance measured at 540 nm.

The control amylase represented 100% enzyme activity and did not contain any sample of analysis. To eliminate the absorbance produced by sample, appropriate extract controls with the extract in the reaction mixture in which the enzyme was added after adding DNS. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the following formula.

$$\text{Activity} = \frac{\text{conc. of Maltose liberated} \times \text{ml of enzyme used}}{\text{mol.wt. of Maltose} \times \text{incubation time (min)}} \times \text{dilution factor}$$

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions.

The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the following formula.

$$\% \text{ inhibition/induction} = \frac{\text{Activity in presence of compound}}{\text{Control activity}} \times 100$$

**Analysis of Acarbose as standard inhibitor:**

Acarbose was used as a standard inhibitor and it was assayed at above mentioned test sample concentrations. The assay method was similar to the above mentioned procedure, instead of test samples, Acarbose was added. The results were compared to that of test sample.

**Anthelmintic activity:**

*Endrullus enguinae* (earth worm) nearly equal size (6 cm) was collected from ICAR Ella–Farm, Old-Goa, Goa.

The anthelmintic activity was performed according to the Ghosh *et al* method[6]. On adult Indian earth worm, *Endrullus enguinae* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Endrullus enguinae* was placed in Petri dish containing three different concentrations (50mg/ml, 100mg/ml, 150mg/ml) of ethanolic extract of *Crossandra infundibuliformis*. Each petri dish was placed with 2 worms and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. In the same manner Albendazole (15mg/ml) was included as standard compound. The test results were compared with standard compound Albendazole treated samples.

**Results and Discussion**

Nature has provided the abundant plant wealth for all the living creatures, which possess medicinal virtues.

Therefore, there is a necessity to explore their uses and to ascertain their therapeutic properties.

**Antidiabetic activity**

The Antidiabetic activity of ethanol extracts of *Crossandra infundibuliformis* was performed at five different concentrations using  $\alpha$ -amylase enzyme inhibition method and the results were presented in the Table 1.

**Table.1. In-vitro antidiabetic activity of the ethanolic extract of *Crossandra infundibuliformis* (ECI) against  $\alpha$ -amylase enzyme.**

SAMPLES	% $\alpha$ -AMYLASE INHIBITION
ECI (100 $\mu$ g)	5.24
ECI (500 $\mu$ g)	22.00
ECI (1000 $\mu$ g)	33.00
ECI (1500 $\mu$ g)	44.53
ECI (2000 $\mu$ g)	51.34
Standard Acarbose (100 $\mu$ g)	100

The sample has displayed significant inhibition of enzyme and could inhibit more than 50% at 2000 µg. Inhibition was also noted at lower volumes as given in the Table.1.

Many herbal extracts are used in Ayurveda for the treatment of diabetes and have been reported to have antidiabetic activity in the inhibition potential towards alpha amylase and glucosidase activity. Alpha amylase and alpha glucosidase are responsible for the hydrolysis of poly and oligosaccharides into monomers or the cleavage of bonds between sugars and non-carbohydrate glycone. Thus, this enzyme is involved in a number of important biological processes, such as digestion of carbohydrate into glucose or processing of the oligosaccharide moieties of glycoprotein.

Currently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents for the treatment of diabetes mellitus. Hence the traditional herbal medicines are mainly obtained from plants are used in the management of diabetes mellitus. In recent years herbal medicines have started to gain importance as a source of hypoglycemic agents. It is estimated that more than thousand plant species are being used as folk medicine for diabetes. Biological actions of the plant products used as alternative medicines to treat diabetes are in relevance to their chemical composition. The literature surveys reveal that phyto-constituents such as alkaloids, glycosides, flavonoids, tannins, steroids and carbohydrate are responsible for the anti-diabetic activity [7]. The presence of these phytoconstituents such as flavonoid, steroids, tannins, carbohydrates, glycosides may be responsible for the anti-diabetic activity of *Crossandra infundibuliformis*. Therefore, intake of *Crossandra infundibuliformis* may help not only in glycaemic control, but also in minimizing the complications associated with diabetes.

### **Anthelmintic activity**

The anthelmintic activity was evaluated on an adult Indian earthworm, *Endrullus enguinae*, due to its anatomical and physiological resemblance with intestinal roundworm parasites of human beings. The progress of Anthelmintic activity of ethanol extracts of *Crossandra infundibuliformis* at three different concentrations: 50mg/ml, 100mg/ml and 150mg/ml in earth worms are presented in Table 2.

**Table.2. In-vitro anthelmintic activity of the ethanolic extract of *Crossandra infundibuliformis*.**

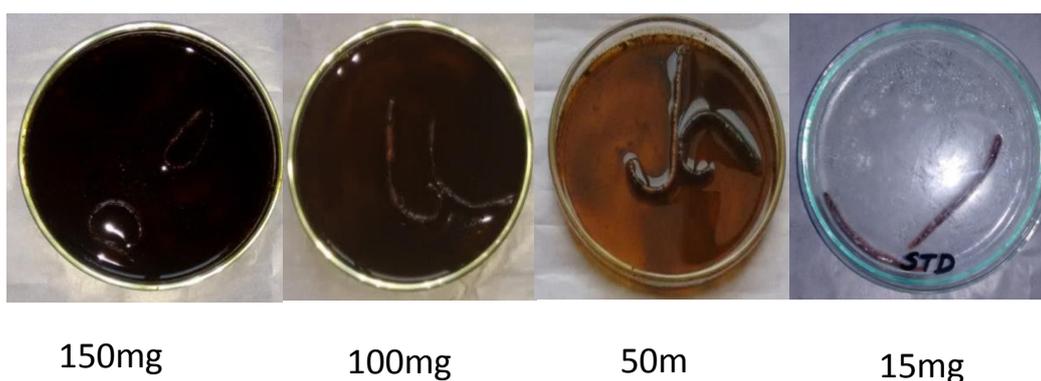
<b>Groups</b>	<b>Drug</b>	<b>Concentration (mg/ml)</b>	<b>Time taken for paralysis (min)</b>	<b>Time taken for death (min)</b>
Group-1	Albendazole (standard)	15mg/ml	37	53
Group-2	Ethanol Extract	50mg/ml	7	11

Group-3	Ethanol Extract	100mg/ml	5	7
Group-4	Ethanol Extract	150mg/ml	3	4

From the Table.2, it was clear that the higher concentration (150mg/ml) of extract produced a paralytic effect much earlier and the time to death was shorter for all worms. The results were compared with that of the standard Albendazole. The ethanol extracts of leaves of *Crossandra infundibuliformis* possessed dose-dependent and significant anthelmintic activity when compared to that exhibited by Albendazole.

In the previous study, we reported the preliminary phytochemical, anticancer and insecticidal activity[8] of the ethanolic extract of the leaves of *Crossandra infundibuliformis*. The presence of phyto-constituents such as Tannins, Steroids, Saponins and Alkaloids may be responsible for the anthelmintic activity[9] of *Crossandra infundibuliformis*.

Tannins produce anthelmintic activity by binding to free protein in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of the parasite and phenolic compounds by uncoupling oxidative phosphorylation hinder the energy production in helminth parasites and may cause death. Alkaloids may act on central nervous system and cause paralysis of the earthworm. The effect would be due to presence of the steroidal alkaloid oligoglycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with its antioxidant effect which is capable of reducing the nitrate generation which could interfere in local homeostasis which is essential for the development of helminthes.



**Figure 1. In-vitro Anthelmintic activity of ethanolic extract of *Crossandra infundibuliformis*.**

## Conclusion

This study suggests that the ethanolic extracts of the leaves of *Crossandra infundibuliformis* possess significant *In-vitro* Antidiabetic and Anthelmintic effect with increasing concentrations. It is anticipated that this plant would be a useful pharmaceutical material to treat Diabetic and Helminthiasis. The present investigations give the evidence that

it may be a fruitful medicine of tomorrow. Future research should focus on the molecular mechanism of *Crossandra infundibuliformis*. There is a need for further investigation of this plant in order to identify and isolate its active principle(s) to treat Diabetes and Helminthiasis.

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