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Research article

In-vitro Anthelmintic activity of *Cassia fistula* extracts on *Pheretima posthuma* model

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Keywords: *Cassia fistula*; Anthelmintic; *Pheretima posthuma, aqueous extract.*

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Abstract

Cassia fistula, commonly known as Golden shower tree, was widely known for its antioxidant, anti-inflammatory, antitumor, hypolipidemic, antimicrobial, hepato-protective activities. Traditionally the plant has been widely used for various infections. The present study involves the phytochemical screening and evaluation of the leaves and flowers of *Cassia fistula* on *Pheretima posthuma* model. The model has been selected as it has a close resemblance with human intestinal round worm, anatomically and physiologically. The plants and earthworm were collected and authenticated. The leaves and flowers of *Cassia fistula* were dried, powdered and subjected for maceration using aqueous solvent. Different concentration of crude extracts, leaves extracts, flower extracts were evaluated for preliminary phytochemical test and anthelmintic activity. All the extracts showed significant anthelmintic activity. Among the extracts, crude leaves extract (40mg/ml) showed better activity than other extracts. The results suggests that *Cassia fistula* may be beneficial in the treatment of parasitic infections.

Introduction

Parasitic diseases are among the most severe and widespread infections in the world, causing millions of morbidities and deaths each year. The global significance of parasitic worms in the gastrointestinal tract cannot be overstated, with the World Health Organization estimating that almost two billion individuals are affected by helminth infections. While synthetic anthelmintic agents are commonly utilized to manage these infections, certain studies have indicated the emergence of anthelmintic resistance, highlighting the urgent need for alternative treatment methods [1]. Synthetic anthelmintic are considered unsafe due to side effects and toxicity, and are often not recommended for young children or pregnant women. The indiscriminate use of anthelmintic medications in treating parasitic disorders has recently been linked to human toxicity, leading to research using plants for the discovery of new anthelmintic compounds. The development of treatment resistance in various hazardous parasites and microorganisms causes massive economic losses due to the scarcity of effective drugs and their high cost. The use of natural items with therapeutic properties is an ancient practice that is gradually gaining popularity [2]. Medicinal plants are regarded as a great source of unique natural compounds and drugs for the development of medicines to treat variety of illnesses and diseases. Many of the anticancer and anti-infective drugs in the drug development are of herbal origin [3].

Cassia fistula, commonly called as golden shower tree belonging to the family Fabaceae, has been traditionally used in Ayurvedic system of medicine as this plant possess unique qualities that are beneficial in treating dermal

infections, inflammatory conditions, ulcers, rheumatism, jaundice as well as anorexia. In addition to treating snakebites, the leaf extracts also work well for cough and ringworm infections [4].

Based on these, various studies has been carried out to explore its pharmacological activities. Extracts from the bark of *C. fistula* Linn. demonstrated notable anti-inflammatory properties in both acute and chronic models [5]. Bhalodia *et.al.* reported significant antibacterial and antifungal activity of hydroalcoholic leaf extracts of *Cassia fistula* [6]. Antitussive, analgesic, antipyretic, wound healing activities, antitumor, antidiabetic, larvicidal and ovicidal activities were also studied using this plant [7-9].

Various parts of this plant has been reported to posses anthelmintic activity against blowfly larvae, *Fasciola gigantic,* sheep gastrointestinal nematodes *etc*. The extracts from seeds and pulp (100 mg/ml) showed significant paralysis and death of worms (*Pheretima posthuma*) compared to Piperazine citrate [10-13].

In view of this, an attempt has been made to study the anthelmintic activity of aqueous extracts of *Cassia fistula* leaves and flowers on *Pheretima posthuma* model, as it has a close resemblance with human intestinal parasite.

Materials and methods

Albendazole used in the study was purchased from local medical shop, Kalpetta.

Collection and authentication of plant material

The leaves and flowers of *Cassia fistula* required for the study were collected from Kalpetta and Rippon of Wayanad, Kerala, during the month of March. The plant species was authenticated by Dr. Raji R, Assistant Professor, Department of Botany, St.Mary's College, Sulthan Bathery, Wayanad.

Preparation of extract

The leaves and flowers were dried under shade and after optimum drying, it was coarsely powdered and stored in air tight container till further use. Fresh leaves required for the preparation of crude extract were collected at the time of study. For the preparation of extract 30 g of leaves and 45 g of flower of *Cassia fistula* were macerated individually using aqueous solvent. After maceration the contents were filtered. The filtrate obtained were allowed to concentrate by placing the marc on a boiling water bath. The dried marc was kept in the desiccator containing silica. The percentage yield of corresponding extracts were determined [14].

Preparation of crude extracts

The leaves required for the preparation of fresh juice were collected on the day of the study. The leaves were washed and cleaned. 20gm of leaves were crushed using mortar and pestle. The juice obtained were filtered and the extracts were dried. The dried extracts were kept in desiccator. The percentage yield of the extract was determined [15].

Preliminary phytochemical analysis

The extracts were screened for the presence and absence of various phytochemicals.

Evaluation of anthelminthic activity

The earthworms collected were washed with saline solution. Distilled water, Albendazole (10mg/ml) and extracts of different concentrations (20mg/ml,40 mg/ml) were taken in different petridish. Earthworms were introduced into each petridish and time taken for paralysis and death of individual earthworms were noted. The paralysis time of each group of earthworms was recorded when no movement was observed besides conditions like vigorous shaking. The time of death were noted when the worms completely stopped moving even when they were shaken vigorously or submerged in warm water having a temperature of approximately 50°C [16].

Statistical analysis

The results were expressed as mean ± SEM(n=6). Statistical analysis was done by one way ANOVA followed by Dunette's multiple comparisons test ($p< 0.05$), using graph pad prism software.

Results

The characteristics of the extracts and the percentage yield were given in (Table 1).

Table 1. Characteristics of Extracts.

Extract	Color	Percentage yield
Leaf extract	Dark brownish	20.93%
Flower extract	Dark brownish	21.18%
Crude extract	Sticky greenish	26.13%

Phytochemical analysis

The phytochemical screening conducted has shown the presence of different constituents in the extracts. Alkaloids, saponins, and tannins were found in all the extracts (Table 2).

Table 2. Phytochemical analysis

Phytochemicals	Aqueous flower Extract	Aqueous leaves Extract	Crude leaves extract
Alkaloids	+	+	+
Carbohydrates	+	-	-
Saponin glycosides	+	+	+
Tannin	+	+	+
Flavanoids	-	+	+
Steroids	+	-	-

Table 3. Anthelmintic activity of aqueous and crude extracts of *Cassia fistula*.

Group	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Distilled water	-	-	-
Albendazole	10 mg/ml	47.08 ± 0.599	78.96±0.89
Aqueous leaf extract	20mg/ml	106.90±2.045	147.7±9.91
Aqueous leaf extract	40mg/ml	62.88±4.204	94.24±2.90
Aqueous Flower extract	20mg/ml	110.70±0.3317	167.8±2.84
Aqueous Flower extract	40mg/ml	68.05±1.413	113.8±3.13
Crude extract	20 mg/ml	100.5±0.787	123.10±6.54
Crude extract	40 mg/ml	58.52±1.67	92.97±6.04

Results were expressed as mean ± SEM, n=6; $p<0.05$, as compared to Albendazole.

The results are given in Table 3. All the extracts showed dose-dependent anthelmintic activity. Crude leaf extract (40mg/ml) showed the maximum effect among all extracts, but it was less potent than albendazole (20mg/ml). All the extracts were found to be statistically significant ($P< 0.05$) when compared to the paralysis time of albendazole.

Discussion

The present study was carried out using crude extract, flowers and leaves extract of *Cassia fistula* on *Pheretima posthuma* model. In this study, *Pheretima posthuma* were selected due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings[17-18]. Earthworms are readily available, which makes them a popular choice for the initial *in vitro* assessment of anthelmintic activity [18].

Albendazole causes death of the parasite, was attributed to its inhibition of tubulin polymerization and blocking glucose uptake [19]. Phytochemical studies show presence of tannins, saponins, flavonoids and alkaloids in all extract. The presence of these compounds may be the reason for the anthelmintic property of the extract of *Cassia fistula*. It has been documented in the literature that plants rich in tannins offer a promising treatment option for helminthic infections. The anthelmintic effects of tannins may be attributed to their ability to bind free proteins required for larval nutrition, consequently reducing nutrient availability and causing larval starvation or stunted growth [20].

Earlier research has also indicated the presence of a significant tannin source in Cassia species, which has been shown to provide protection against various parasitic worms, which is in correlation with our study.

Conclusion

The result of the present study shows that fresh juice, leaves and flower extracts of *Cassia fistula* has the potential to use as anthelmintic against *Pheretima posthuma*. Further studies are needed to explore the mechanism as well as the principle compound responsible for the activity.

Ethics Approval and Consent to Participate

Not applicable.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this publication.

Author Contribution

All authors are contributed equally in the research

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Data availability

All data generated or analyzed during this study are included in this published article.

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