



## IN VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF *CAESALPINIA PULCHERRIMA* (LINN) FLOWER EXTRACTS IN INDIAN EARTHWORM

Dhaked P.S. \*, Panigrahy R.N, Kshirsagar S.N.

Department of Pharmacology, Sudhakar Rao Naik Institute of Pharmacy, Pusad (M.S.) 445204 India.

\*Corresponding author's E-mail: [p.s.dhaked@gmail.com](mailto:p.s.dhaked@gmail.com)

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### ABSTRACT

*Caesalpinia pulcherrima* (Linn) Popularly known as Peacock flower in India belongs to family Caesalpinaceae. The objective of the present work was to evaluate the in-vitro anthelmintic potency of the ethanolic, petroleum ether and aqueous extract of *Caesalpinia pulcherrima* (Linn.) flower (CPF) using Indian earthworms (*Pheretima posthuma*). The various concentrations (30, 50, 70 and 100mg/ml) of extracts were tested in-vitro for anthelmintic potency by determination of time of paralysis and time of death of worm. Piperazine citrate (15mg/ml) used as standard. Present study indicates that *Caesalpinia pulcherrima* (Linn.) potentiate to paralyze earthworm and also caused its death after some time. The results of the study are comparable to standard Piperazine citrate. The result showed that aqueous extracts took less time to cause paralysis of the earthworm than that of ethanolic extract and pet. Ether extract. Thus the present study demonstrate that the traditional claim of *Caesalpinia pulcherrima* (Linn.) as an anthelmintic has been confirmed as the Aqueous, ethanolic, and petroleum ether extracts displayed activity against the earthworm used in study.

**Keywords:** *Caesalpinia pulcherrima* (Linn) flower, anthelmintics, Paralysis, Death of earthworm.

### INTRODUCTION

Parasitic helminthes affects animals and men, causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic; debilitating nature they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The major control strategy adopted against helminthes parasite is the use of anthelmintics.<sup>1</sup> *Caesalpinia pulcherrima* (Linn.) Swartz (Leguminosae) commonly known as red bird of paradise is a medicinal herb used in the treatment of various diseases.<sup>2</sup> The different parts of this herb have been used in common remedies for treatment of a number of disorders including pyrexia, menoxenia, wheezing, bronchitis and malarial infection.<sup>3</sup> The plant is rich in many pharmaceutical active ingredients like flavonoids, aritonoids, glycosides and sterols.<sup>4</sup>

Traditionally *Caesalpinia pulcherrima* (Linn.) claim as anthelmintic but scientifically it is not revealed yet thus the present study was design to evaluate the in-vitro anthelmintic activity of petroleum ether, ethanolic and water extract of *Caesalpinia pulcherrima* (Linn.) flower.

### MATERIALS AND METHODS

**Plant:** The fresh flowers of *Caesalpinia pulcherrima* (linn.) were collected in the month of September 2010 from its natural habitat at mudkhed village in Nanded region, Maharashtra, India. The plant was authenticated by Dr. A. Chaurvedi of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen (No: 9439) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

**Experimental worms:** All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity.

**Material:** Petroleum ether Ethanolic and Water extracts of *Caesalpinia pulcherrima* (Linn.) flower, Piperazine citrate (GSK. Ltd, Mumbai).

**Preparation of Extracts of *Caesalpinia pulcherrima* (Linn.) flower:** The *Caesalpinia pulcherrima* (Linn) Flowers were dried under shade and undergone crushing in electric blender to form powdered and subjected to successively extraction by Pet. ether and ethanol using Soxhlet's extractor. The percent yield of ethanolic extract was 24.8% w/w and petroleum ether (60 Grade) extract yield 6.1% w/w. Both the extracts were concentrated by evaporation at room temperature and were used for pharmacological studies. Powdered material of *Caesalpinia pulcherrima* (Linn.) flower was kept for maceration with 1000 ml of distilled water for 7 days. The Aqueous extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be 23.56 percent.

### Administration of Extract

Different concentrations of both extracts of CPF (30-100mg/ml) were prepared by using 0.2% v/v of tween-20 as a suspending agent and final volume was made to 10 ml for respective concentration. Piperazine citrate (15mg/ml) was prepared similar to extract and used as standard.



## Experimental Design

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity. Earthworms were divided into fourteen groups (5 each). The first group (I) served as normal control which received saline water only. The second (II) group received the standard drug is Piperazine citrate at a dose level of 10 mg/ml. Groups (III) to (IV) received doses of Pet. ether extracts of 30 mg/ml, 50 mg/ml, 70 mg/ml, 100 mg/ml, respectively. Groups (VII) to (X) received doses of ethanolic extracts of 30 mg/ml, 50 mg/ml, 70 mg/ml, 100mg/ml, respectively and Groups (X) to (XIV) received doses of aqueous extracts of 30 mg/ml, 50 mg/ml, 70 mg/ml, 100 mg/ml, respectively.

The anthelmintic assay was carried out as per the method of Ajaiyeoba et.al, 2001<sup>5</sup> with minor modification.<sup>6,7</sup> Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when shaken vigorously nor when dipped in warm water (50<sup>o</sup>c), followed with their body colors fading away)<sup>8,9</sup>. All the results were expressed as Mean  $\pm$  S.D of five worms in each group.

### Anthelmintic effect of CPF in Indian adult earthworms (*Pheretima posthuma*)

Fourteen groups of approximately equal size worms consisting of five earthworms individually in each group were released into 10 ml of desired concentration of standard drug and extracts of CPF in Petri plate.

## RESULTS AND DISCUSSION

Helminthiasis or infections with parasitic worms are pathogenic for human beings. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelmintics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues<sup>10,11</sup>.

The extracts of *Caesalpinia pulcherrima* (Linn.) flower produced a significant anthelmintic activity in dose dependent manner as shown in Table 1.

The Anthelmintic activity of aqueous, ethanolic and Pet. ether extract were comparable with that of standard drugs. The 0.5% CMC Solution in distilled water was used as a control. The activity shown by aqueous, ethanolic and Pet. ether extracts is of considerable importance and has justified its use in controlling the disease causes by worms as reported by the tribal people. By employing one-way ANOVA, all data were found to be statistically significant at 5 % level of significant ( $p < 0.05$ ). The extent of activity shown by the crude extracts was found to be better than that of the standard drug Piperazine citrate which justifies its activity as shown in Fig 1.

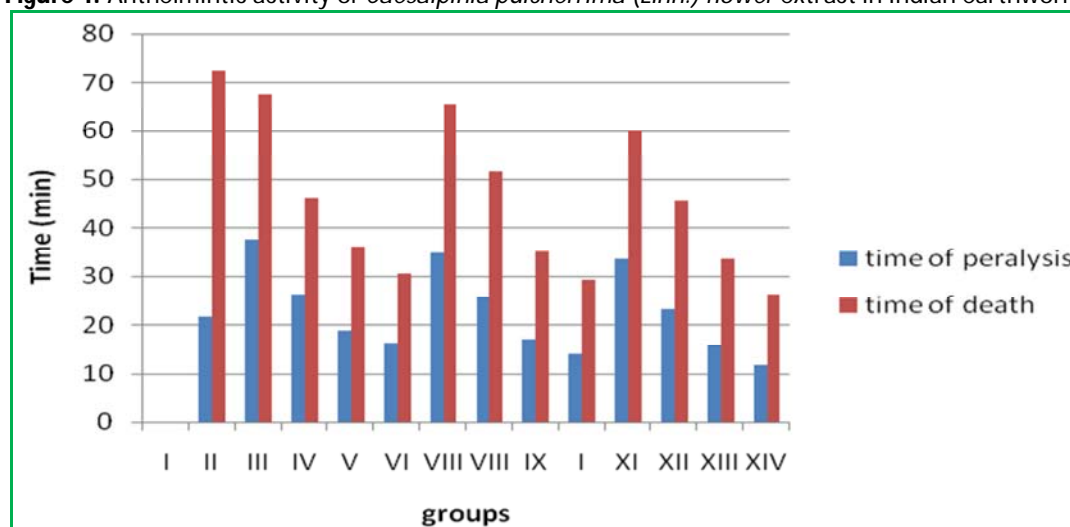
It could be concluded and confirmed that the aqueous, ethanolic and Pet. ether extract of flower of plant *Caesalpinia pulcherrima* (Linn.) is having anthelmintic activity. Aqueous extract showed better anthelmintic activity in comparison to the ethanolic and Pet. Ether extract of *Caesalpinia pulcherrima* (Linn.). Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity.

**Table 1:** Anthelmintic Potency of *Caesalpinia Pulcherrima* (Linn) Flower.

Drug Treatment	Groups	Concentration (Mg/ml)	Time of paralysis (min) (Mean $\pm$ S.D)	Death of paralysis (min) (Mean $\pm$ S.D)
Control	I	-	-	-
Piperazine citrate (Std.)	II	20	21.66 $\pm$ 2.18	72.33 $\pm$ 2.054
Pet. Ether extract of <i>Caesalpinia pulcherrima</i> (Linn.)	III	30	37.87 $\pm$ 2.64	67.64 $\pm$ 3.12
	IV	50	26.19 $\pm$ 1.89	46.33 $\pm$ 1.98
	V	70	18.89 $\pm$ 3.43	35.97 $\pm$ 2.26
	VI	100	16.36 $\pm$ 4.06	30.53 $\pm$ 2.54
Ethanolic extract of <i>Caesalpinia pulcherrima</i> (Linn.)	VII	30	34.67 $\pm$ 4.03	65.51 $\pm$ 1.42
	VIII	50	25.76 $\pm$ 1.67	51.62 $\pm$ 3.24
	IX	70	17.03 $\pm$ 2.42	35.02 $\pm$ 4.79
	X	100	14.07 $\pm$ 1.66	29.08 $\pm$ 3.87
Aqueous extract of <i>Caesalpinia pulcherrima</i> (Linn.)	XI	30	33.45 $\pm$ 3.50	59.86 $\pm$ 2.34
	XII	50	23.01 $\pm$ 1.07	45.65 $\pm$ 1.65
	XIII	70	15.86 $\pm$ 1.93	33.42 $\pm$ 1.22
	XIV	100	11.57 $\pm$ 1.78	25.16 $\pm$ 2.47

Each value represents the mean  $\pm$  SD ( $n = 5$ )



**Figure 1:** Anthelmintic activity of *Caesalpinia pulcherrima* (Linn.) flower extract in Indian earthworm.

Each bar is represented as mean  $\pm$  standard deviation (n = 5)

Thus from results the traditional claim of *Caesalpinia pulcherrima* (Linn.) as an anthelmintic have been confirm as a leaves extracts displayed activity against the worm used in present study. The possible mechanism of the anthelmintics activity of *Caesalpinia pulcherrima* (Linn.) cannot be explained on the basis of our present results. However, it may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis<sup>12, 13</sup>. While there need further study to isolate and revealed the active compound contained in the crude extract of *Caesalpinia pulcherrima* (Linn.) as well as to establish mechanism of action.<sup>14</sup>

## REFERENCES

1. Kapoor LD, Hand book of ayurvedic medicinal plants:Herbal reference Library, USA, Florida, CRC Press , 2000, p 82.
2. Srinivas KVNS, Rao YK, Mahender I, Flavonoids from *Caesalpinia pulcherrima*, Phytochemistry, 2003, 63, 789-793.
3. Chiang LC, Chiang W, Liu WC, Lin CC, In vitro antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids, J. antimicrob Chemother, 2003, 52, 194-198.
4. Krag K, Plants used as contraceptives by the North American Indians: an ethnobotanical study, Botanical Museum, Cambridge, MA: Harvard University (1976).
5. Ajayeoba EO, Oncha SA, Olarenwaju OT, In vivo anthelmintic property of Buehholria cariacen & Gyandropis gyancha ext., Pharm Biol., 2001, 39, 127-210.
6. Guha P, Betel leaf: The neglected green gold of India. J. Hum Ecol., 2006, 19(2).
7. Chopra RN, Nayer SC, Chopra IC, Glossary of Indian medicinal plants. CSIR, NewDelhi, 1956; 160.
8. Kelly JD and Hall CA, Resistance of animal helminthes to anthelmintics, Adv. Pharm.Ther ., 1979, 16, p S9-S12.
9. Dash OK, Suresh P, Kar DM, Ganpaty S, Panda SP, Evaluation of *Evolvulus alsinoids* Linn, for Anthelmintics and antimicrobial activities, J N at SRem., 2002, 2, p.182-185.
10. Jain MN, Jain SR, Therapeutic activity of *Ocimum basilicum* var. album,Planta Med., 1972, 22, p.66-70.
11. Okon ED, Ogunsusi RA, Fabiyi JP, Survey and feasibility studies on fascioliasis and parasitic gastroenteritis of ruminants in Nigeria, Federal Livestock Department of Nigeria Report. 1980
12. Singh D, Swarnkar CP, Khan FA, Anthelmintic resistance in gastrointestinal nematodes in livestock in India. J Vet Parasit 2002, 16:115-130.
13. Goodman LS Gilman A, The Pharmacological basis of Therapeutics. 10th Edn. Mcgraw Hill Medical Publishing Division, New York, 2001; 1121.
14. Tripathi KD, Essential of Medical Pharmacology, 4th Edn. Jaypee Publication, New Delhi, 2006; 816-817.

## About Corresponding Author: Mr. Dhaked Pushpendra Singh



Mr. Dhaked Pushpendra Singh Graduated at Department of pharmaceutical science of Mohanlal Shukhadia University, Udaipur, Rajasthan, India and Currently Doing post graduation in Pharmacology from Sudhakarroa Naik Institute of Pharmacy Pusad, in Amravati University, Maharashtra, India.