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INVESTIGATION OF *IN VITRO* ANTHELMINTIC ACTIVITY OF *CAESALPINIA PULCHERRIMA* LEAVES

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Abstract

The crude Pet. Ether, dichloromethane, ethyl acetate and ethanol extracts of *Caesalpinia pulcherrima* (Caesalpiniaceae) leaf were investigated for *in-vitro* anthelmintic activity on the Indian adult earthworms *Eisenia foetida*. The various concentrations (20, 40, 60mg/ml) of extracts were tested *in-vitro* for anthelmintic potency by determination of time of paralysis and time of death of worm. The leaf extracts of *Caesalpinia pulcherrima* exhibited a dose dependant inhibition of spontaneous motility (Paralysis) of earthworms. Piperazine citrate (10mg/ml) was used as standard drug and distilled water containing 2% tween 80 was as control. All the extracts were found to be exhibited dose dependent anthelmintic activity. The decreasing order of activity of extracts was ethyl acetate, ethanol, dichloromethane and petroleum ether extracts. Thus the present study demonstrates that the leaf of *Caesalpinia pulcherrima* could be categorized under anthelmintic herbal drugs and could be used as a potent key ingredient of herbal formulation.

Key words: *Caesalpinia pulcherrima,* Anthelmintics, *Eisenia foetida*, Piperzine citrate.

Introduction

Helminth or worm infection is one of the major worldwide public health problems, more in tropical nations. Helminthes infections are being recognized as a cause of much acute as well as chronic illness among the various human beings as well as animals. Anthelmintic or antihelminthics are drugs that expel parasitic worms (helminths) from the body, by either staggering or killing them (Khadse *et al.,* 2010). The majority of drugs available to treat these infections possess some common side effects like nausea, vomiting, abdominal pain, expulsion of ascaris from mouth or nose, allergic reactions, loss of hair, urticaria, granulocytopenia, fall in blood pressure, sedation, fever, body ache etc (Tripathi, 2005). Therefore, there is a scope for search of new drugs especially from the herbal origin, which are known to possess negligible side effect and better potency.

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Plants belonging to the family Caesalpiniaceae have wide folklore medicinal uses. *Caesalpinia pulcherrima* popularly known as peacock flower is widely cultivated in gardens throughout India (Wealth of India, CSIR, 1983, Khare, 2007). Plant is used as emmenagogue, purgative, stimulant and abortificient. In eastern India the leaves are used as a substitute for senna. The different parts of this plant like bark, flower, leaves have been used in common remedies for treatment of a number of disorders including pyrexia, menoxenia, wheezing, bronchitis, antiviral and malarial infection. The bark is used as an abortifacient and an infusion of leaves is used as abortifacient and cathartic (Khare, 2007, Kirtikar *et al.,* 1984, Chiang *et al.,* 2003). The plant contains a ûavonoid, myricitroside. fruits contain tannins, gums, resin, benzoic acid. Presence of cyaniding 3, 5-diglucoside is also reported from the ûowers, hydrocyanic acid from the leaves. The root contains caesalpin type diterpenoids along with sitosterol. The leaves have displayed anticancer

activity in laboratory animals. A diterpenoid, isolated from the root, also showed anticancer activity. In Pakistan, the leaf and ûower extract exhibited activity against Gram positive bacteria (Khare, 2007). The leaves, flowers and the plant is rich in many pharmaceutical active ingredients like flavonoids, carotinoids, glycosides, phenols and steroids. The stems contain a cassane-type diterpene ester, pulcherralpin, peltogynoids, bonducellin and 6-methoxypulcherrimin, homoisoflavonoids (Chakraborthy *et al.,* 2009). The objective of the present work is to investigate the anthelmintic activity of pet. ether, dichloromethane, ethyl acetate and ethanol extracts of *Caesalpinia pulcherrima* leaf on worms.

Materials and Methods

Collection of plant and Authentication

The leaves of *Caesalpinia pulcherrima* were collected from Dindigul, Tamil Nadu, India, during the month of August, 2009. The botanical identity of the plant was confirmed by Regional Research Institute (Ay.), Bangalore, India. A voucher specimen (RRI/BNG/SMP/ Drug Authentication/2009-10/554) has been deposited at the Museum of the Department of Pharmacognosy, Lovely School of Pharmaceutical Sciences, Phagwara, Punjab, India.

Preliminary phytochemical investigation

Phytoconstitutents were detected by applying qualitative chemical tests on all extracts of *Caesalpinia pulcherrima* L. leaf.

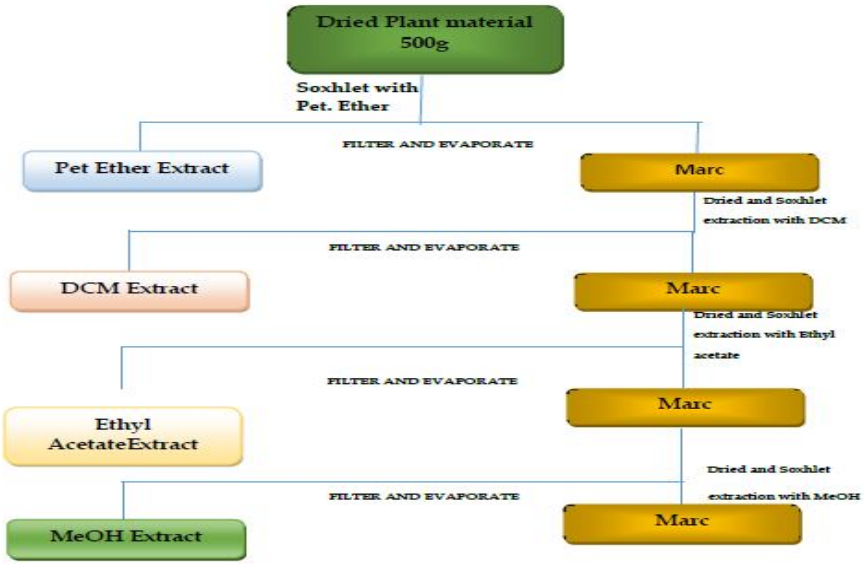
Preparation of extract: The authenticated aerial parts were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. Four extracts were used *viz*. petroleum ether, DCM, ethyl acetate and ethanol extracts which were prepared by adopting the successive solvent extraction method using the Soxhlet apparatus (Fabricant. *et al.,* 2001, Suttee *et al.,* 2016). Different concentrations of all extracts (20, 40, 60 mg/ml) were prepared with the help of distilled water containing 2% tween 80. All the concentrations were evaluated for anthelmintic activity. Piperazine citrate of concentration 10mg/ml was prepared similarly and used as standard.

Table 1: Allocation of earthworms to various groups.

Group no.	Group name	Dose (mg/ml)
i.	Control group (vehicle)	-
ii.	Standard group (Piperazine citrate)	10
iii.	Test group (Pet. Ether extract)	20
iv.	Test group (Pet. Ether extract)	40
v.	Test group (Pet. Ether extract)	60
vi.	Test group (DCM extract)	20
vii.	Test group (DCM extract)	40
viii.	Test group (DCM extract)	60
ix.	Test group (Ethyl acetate extract)	20
x.	Test group (Ethyl acetate extract)	40
xi.	Test group (Ethyl acetate extract)	60
xii.	Test group (Ethanol extract)	20
xiii.	Test group (Ethanol extract)	40
xiv.	Test group (Ethanol extract)	60

Experimental Model

Adult Indian earthworms, *Eisenia foetida,* having anatomical and physiological resemblance with intestinal roundworm parasite of the human being, (David *et al.,* 1983, Shivkumar *et al.,* 2003) were used to evaluate anthelmintic activity. These were collected from moist soil and washed with normal saline to remove all faecal matter. The earthworms were authenticated from Ujjwal Ujala Vermi Group, Amritsar.



Schematic diagram of extraction

Anthelmintic activity

The anthelmintic assay was carried out as per the method of (Kosalge *et al.,* 2009, Ajaiyeoba *et al.,* 2001, Vigar *et al.,* 1984, Gbolade *et al.,* 2008, Athnasiaduo *et al.,* 2001, Thompson *et al.,* 1995, Martin *et al.,* 1997) Petridishes of equal size were taken and numbered. Six earthworms of similar sizes were placed in each petridish as indicated in table 1. Piperazine citrate (10mg/ml) was used as a reference

Table 2: Effects of control and standard drug on earthworms.

Conc. (mg/ml)	Control		Standard	
	Paralysis time (min.)	Death time (min.)	Paralysis time (min.)	Death time (min.)
10	-	-	30.3±0.88	80.67±0.67

Table 3: Effects of *C. pulcherrima* leaf extracts on earthworms.

Conc. (mg/ml)	Petroleum extract		DCM extract		Ethyl acetate extract		Ethanol extract	
	Paralysis time (min.)	Death time (min.)	Paralysis time (min.)	Death time (min.)	Paralysis time (min.)	Death time (min.)	Paralysis time (min.)	Death time (min.)
20	21±0.58**	481.67±0.88**	122.3±1.45**	210.67±0.67**	8.33±0.88**	13.67±0.88**	62.7±1.45**	76±1.0**
40	18±0.58**	361±1.0**	32.3±1.45	165±0.58**	5±0.58**	9.33±0.67**	16.8±1.64**	22±0.58**
60	15.67±0.67**	301.67±0.88**	20.3±0.88**	119.67±0.33**	3.5±0.29**	5.5±0.29**	13.5±0.29**	21.67±0.88**

Significant at *P<0.05, **P<0.01 (One way ANOVA, Dunnet: compare all *vs.* standard applied) Standard *vs*. low, medium and high doses of CP. Values are mean ± SEM, n = 3. CP- *Caesalpinia pulcherrima*.

standard and distilled water containing 2% tween 80% as a control. Observations were made for the time taken for paralysis and death of worms. Paralysis was said to occur when worm did not revive warm water. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C), followed with fading away of their body colours.

Preparation of doses

Test samples of all four extracts were prepared at the concentration, 20, 40 and 60 mg/ml in 25ml of distilled water containing 2% Tween 80.

Statistical Analysis

Each group consisted of 6 earthworms. The readings were taken in triplicate. The data was reported as mean ± SEM (N=3). Evaluation of anthelmintic activity was done by comparing with reference standard Piperazine citrate using ANOVA followed by Dunnet test P<0.05 was considered statistically significant.

Result and Discussion

Preliminary Phytochemical screening

The phytochemical screening revealed that Pet. Ether, DCM, ethyl acetate and methanol extracts contain gums and mucilage, flavonoids, alkaloids, steroids, tannins, glycosides, diterpenes, amino acids and saponins.

The extracts of *Caesalpinia pulcherrima* (L.) leaf produced a significant anthelmintic activity in dose dependent manner as shown in table 2 and 3. Ethyl acetate extract was most effective in causing death of earthworms at all concentrations. The decreasing order of anthelmintic activity of different extracts taken comes out to be-ethyl acetate >ethanol > DCM > petroleum ether extracts. Ethyl acetate extract exhibits better anthelmintic activity than the standard. In the case of petroleum ether extract, paralysis was caused earlier but death time was longer. In the case of DCM extract, the paralysis time was longer at lower dose (20 mg/ml) but shorter at higher doses (40-60 mg/ml). The death time was long but shorter than that of petroleum ether extract. In case of ethyl acetate and ethanol extracts, paralysis and death times were nearby at all doses.

Conclusion

It could be concluded that both ethyl acetate and ethanol extracts of *Caesalpinia pulcherrima* (L.) leaves possess potent anthelmintic activity. Thus *Caesalpinia pulcherrima* (L.). leaf can be used in controlling the diseases caused by worms. But 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 and to establish the effectiveness and pharmacological rationale for the use of *Caesalpinia pulcherrima* as an anthelmintic drug.

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