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STUDY OF *IN VITRO* ANTHELMINTIC ACTIVITY OF *CAESALPINIA BONDUC* LEAVES

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ABSTRACT

The *in vitro* anthelmintic activity of *Caesalpinia bonduc* leaves was evalauated. Four extracts viz. Petroleum ether, Dichloromethane (DCM), Ethyl acetate and Ethanol extracts of *Caesalpinia Bonduc* leaves were investigated for the anthelmintic activity against Earthworms (*Eisenia foetida*). Three concentrations (20, 40, 60mg/ml) of each extract were studied which included the determination of time of paralysis and time of death of earthworms. Piperazine citrate (10mg/ml) was used as standard drug and distilled water containing 2% Tween 80 was used as control. All the extracts exhibited dose dependent anthelmintic activity. The decreasing order of activity of extracts was ethyl acetate, ethanol, DCM and petroleum ether extracts. An Ethyl acetate extract of plant leaf exhibited in vitro anthelmintic activity in a concentration range of (20-60mg/ml). Conclusively, ethyl acetate extract of *Caesalpinia bonduc* leaves possesses vermicidal activity and found to be effective as an anthelmintic.

Keywords: *Caesalpinia bonduc, Eisenia foetida,* anthelmintic, piperazine citrate.

INTRODUCTION

There are about 60 Species in the genus *Caesalpinia* which are tropical and subtropical and widely used for medicinal purposes. This genus contains mostly trees or climbing shrubs. In this study, anthelmintic activity of one species of this genus viz. *Caesalpinia bonduc* has been evaluated. *Caesalpinia bonduc* is a large, thorny, straggling, shrub which behaves like a strong woody climber, taking support of trees. The branches are armed with hooks and straight hard yellow prickles. Leaves are large, double compound, with 7 pairs of pinnae, and each with 3-8 pairs of leaflets with 1-2 small recurved prickles between them on the underside. Flowers are yellow, in dense long-stalked racemes at the top. Fruits are inflated pods, covered with prickles. Seeds are 1-2 per pod, oblong or globular, hard, grey with a smooth shiny surface. The hard and shiny seeds are green, turning grey (Kirtikar and Basu, 1999).

Leaves and seeds after roasting with castor oil are applied externally to inflammatory swellings especially to inflamed piles (Kirtikar and Basu, 1999).

Common names include fever nut, bonduc nut (Kirtikar and Basu, 1999**)**. The chemical constituents present in it are flavonoids, terpenoids, alkaloids, glycosides, phenols, tannins and phytosterols.

MATERIALS AND METHODS

Collection of plant material and its identification

The leaves of *Caesalpinia bonduc* 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, Bangalore, India. A voucher specimen (RRI/BNG/SMP/Drug Authentication/ 2009-10/552) has been deposited at the Museum of the Department of Pharmacognosy, Lovely School of Pharmaceutical Sciences, Phagwara, Punjab, India.

Traditional uses: The leaves are used traditionally as emmenagogue, abortifacient, laxative, purgative and cathartic. The flowers are used as anthelmintic and in the treatment of cough and catarrh (Khare, 2007).

Extracts used: 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.

Organism used: Adult same age Earthworms (*Eisenia foetida*) were used and were procured and authenticated from Ujjwal Ujala Vermi Group, Amritsar.

Anthelmintic activity: Test samples of all the four extracts were prepared in the concentrations, 20, 40 and 60 mg/ml in 25ml of distilled water containing 2% Tween 80. Six earthworms of approximately same size were placed in petridish (diameter 9cm) containing above solution of extracts. Piperazine citrate (10mg/ml) was used as standard drug and distilled water containing 2% Tween 80 was used as control. Anthelmintic activity of Piperazine citrate mediates through hyper polarization that leads to muscle relaxation and flaccid paralysis (Martin, 1985). Time for paralysis was noted when no movement of any sort could be observed except when the

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worms were shaken vigorously. Time of death for worms was noted when the earthworms neither moved when shaken vigorously nor when dipped in warm water 50˚C (Kosalge, 2009). Indian earth worms resemble intestinal round worm parasite of human beings (Vidyarthi, 1967; Chatterjee, 1967). Tannins produce anthelmintic activity by binding to free protein in the gastrointestinal tract of the host animal (Athnasiaduo, 2001) or glycoprotein on the cuticle of the parasite (Thompson, 19950 and phenolic compounds by uncoupling oxidative phosphorylation hinder the energy production in helminth parasites (Martin, 1997). Phytochemical analysis of leaves of *C. Bonduc*revealed the presence of tannins as one of the constituent.

RESULTS AND DISCUSSION

As reported in the tables 1 and 2. All the extracts exhibited dose dependent anthelmintic activity against earthworms. *C. bonduc* leaf extracts showed significant effects ($P<0.001$) at the tested concentrations (20-60mg/ml) as determined by the paralysis and death time (Table 1and 2). 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 howver death time was longer. In the case of DCM extract, the paralysis time was longer at lower dose (20mg/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 nearly similar to all doses. Further study is to be done to determine the mechanism involved and constituent responsible for anthelmintic property.

Table 1. Effects of control and standard drug on earthworms (Gbolade, 2008).

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 2. Effects of *C. bonduc* leaf extracts on earthworms (Gbolade, 2008).

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 followed by Dunnet test: compare all vs. standard applied) Standard vs. low, medium and high doses of CP. Values are mean ± SEM, n = 3. CP- *Caesalpinia bonduc*

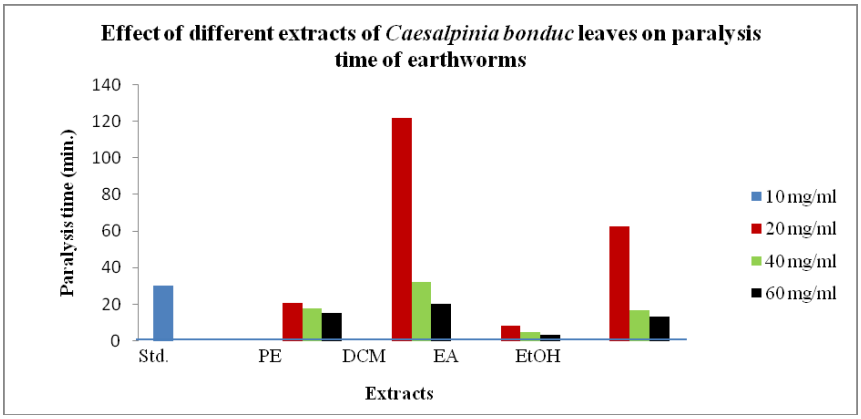

Fig. 1. Graph showing effect of different extracts of *Caesalpinia bonduc* leaves on paralysis time of earthworms. PE- Petroleum ether, DCM- Dichloromethane, EA- Ethyl acetate, EtOH- Ethanol, Std.- Standard (Piperazine citrate).

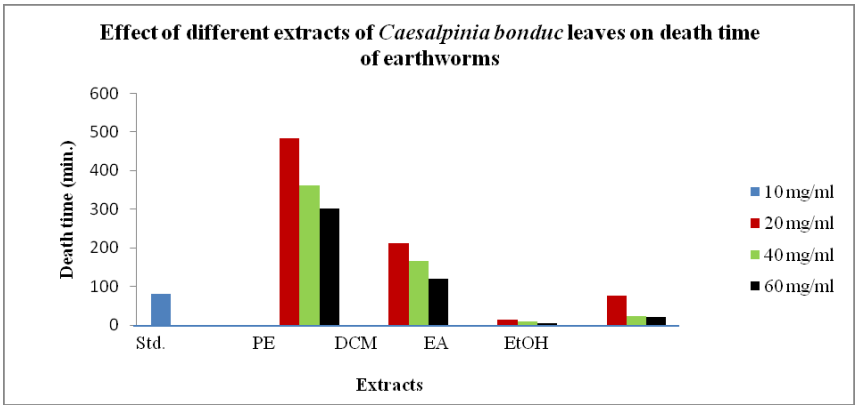

Fig. 2. Graph showing effect of different extracts of *Caesalpinia bonduc* leaves on death time of earthworms.
PE- Petroleum ether, DCM- Dichloromethane, EA- Ethyl acetate, EtOH- Ethanol, Std.- Standard (Piperazine citrate)

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