

**IN VITRO ANTIFILARIAL ACTIVITY OF
Caesalpinia bonduc (L) Roxb. AGAINST
MICROFILARIAE OF *Wuchereria bancrofti* AND
MACROFILARIAE OF *Setaria digitata*.**

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Summary

A preliminary laboratory trail was performed to evaluate antifilarial activity (*invitro*) of *Caesalpinia bonduc* (L) Roxb. leaf extracts (Petroleum ether, ethanolic, aqueous) and fixed oil from seeds, against the microfilariae of *Wuchereria bancrofti*, an human parasite and macrofilariae of *Setaria digitata*, an animal parasite. The mortality of the microfilariae of *W. bancrofti* over the 24 hr observation period in 96 well micro titre plate was used a measure of the activity, whereas the time for paralysis and death were used as a measure of the activity for the animal parasite, *S. digitata*. Ethanolic extract of leaf and fixed oil seeds demonstrated the strongest activity against both the parasites i.e., animal and human parasite. These findings provide a pharmacological basis for the folkloric applications for elephantiasis and hydrocele of this plant.

Key Words: Antifilarial, *Wuchereria bancrofti*, *Setaria digitata*, *Caesalpinia bonduc*

Short title: Antifilarial activity of *Caesalpinia bonduc* (L) Roxb.

Introduction

Among all the tropical diseases, lymphatic filariasis is the most prevalent one. Lymphatic filariasis is the most debilitating, disfiguring scourge, which is estimated to be one of the leading causes of disability worldwide. In humans it is caused by several coiled, round thread like parasite worms like *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori* and in animals it is caused by *Setaria digitata*. The available interventions for filaria have significant limitations as they require annual repeated treatments for much number of years and drug resistance may become a critical issue after long treatment. Hence there is a need for the development of new drugs or combinations either to treat or to suppress the filarial parasite^{1,2,3}. The use of natural substances, particularly plants in disease control is being practiced from centuries, which led to the discovery of many modern pharmaceuticals⁴. The ethno medical information reveals that number of plants were used in the treatment of elephantiasis, one among them is *Caesalpinia bonduc* (L) Roxb^{5,6,7}. The present study reports the antifilarial activity of *C.bonduc* against *W.bancrofti* and *S.digitata*.

Material and Methods

Caesalpinia bonduc

The seeds of *C. bonduc* were collected at Alagar koil hills of Madurai district, India. The leaves and seeds were authenticated at Department of Botany, The American College, Madurai and a voucher specimen has been filed in Department of Pharmacognosy, Madurai Medical College, Madurai, India.

Preparation of extracts

The shade dried leaves of *C. bonduc* were powdered and extracted with petroleum ether (60-80°C) and ethanol individually by cold maceration procedure.

The aqueous extract was prepared by refluxing the dried leaves with water. The crude extracts were evaporated to dryness and the residues (Petroleum ether extract- 6.85 % w/w, ethanolic extract-3.97 %, aqueous extract- 7.15 %) were maintained in refrigerator until used for the study.

Pale, yellow, viscous oil having disagreeable odour (21.7 %) was extracted from the decorticated seeds of *C. bonduc* using petroleum ether (60-80°C) in soxhlet apparatus for 6 hours.

Isolation of worms

W.bancrofti^{8,9}

Microfilariae were isolated from the blood samples of positive carriers as described by Devaney and Howells¹⁰, with few modifications. Blood samples were diluted with equal volume of normal saline and filtered through 5µ nucleopore membrane filter. After passing two more volumes of normal saline, the membrane was removed and transferred to petridish, washed thoroughly with normal saline and the washings were centrifuged at 2000g for 10 minutes. The supernatant was discarded and the microfilariae were maintained in RPMI 1640 medium (Sigma St Louis). The collected worms were authenticated as microfilariae of *W. bancrofti* by the Medical Entomologist, Institute of Microbiology, Madurai Medical College, Madurai, India.

RPMI 1640 medium was prepared by dissolving RPMI 1640 culture medium (1 liter packet), 10 % Human serum, 15 mM HEPES, Gentamycin (20 mg), Penicillin (100 mg), Streptomycin (100 mg), Sodium bicarbonate (2 g) in 1 liter of sterile triple distilled water and sterilized by filtration through 0.22 µ membrane filter.

S.digitata

Mobile adult worms of *S. digitata* having average length of 6.0 ± 1 cm, average weight of 35 ± 6 mg were collected from the freshly slaughtered cattles and brought to the laboratory in modified ringer's solution and authenticated at the

Department of Veterinary Parasitology, Veterinary College and Research Institute, Nammakal, Tamil Nadu, India.

Experimentation

Microfilaricidal activity

The various extracts of leaf and fixed oil from seeds were diluted with RPMI 1640 culture media to obtain concentrations of 25, 50 and 100 µg/ml. Microfilariae were incubated in the test solution containing the leaf extracts, fixed oil and as control, microfilariae were incubated in the RPMI 1640 medium without any extract. All assays used 10 microfilariae and were in triplicate. The microfilaricidal activity was carried out in 96 well micro titre plate, which was incubated at 37°C in 5% CO₂ atmosphere and the mortality was recorded after 24 hours.

Macrofilaricidal activity¹¹

Test solutions of the various extracts for the evaluation of macrofilaricidal activity were prepared in three different concentrations (25, 50 & 100 mg/ml) by dissolving the extracts in 1 % gum acacia in modified ringer's solution. Two worms in triplicate were placed in petridish with solution of various extracts in all three different concentrations. Time for paralysis (P in minutes) was taken when no movement of any kind could be observed, except when the worms are moved vigorously. Time for death (D in minutes) of the worms were captured after confirming that the worms neither moved when shaken vigorously nor when immersed in warm water (50°C). Albendazole in a concentration similar to that of the test solution was included as standard drug, while modified ringer's solution was included as control.

Results & Discussion

Microfilaricidal activity

Effect of leaf extract and fixed oil against microfilariae of *W.bancrofti* were shown in Table 1 & 2. All control microfilariae gave 0% mortality after 24 h incubation at 37°C in 5 % CO₂ atmosphere showing the control microfilariae were not affected. The percentage mortality was calculated and corrected mortality was obtained by Abbots formula. All the extracts showed a dose dependent antifilarial effect. The higher the concentration, the more the effect of extracts towards the microfilariae. The mortality recorded in ethanolic extract of leaf and fixed oil from the seeds were promising. The percentage of mortality recorded in ethanolic extract of leaf were 30, 53.3, and 80 at 25, 50, 100 µg/ml respectively and in the fixed oil treated were 46.6, 66.6 and 80 at 50, 100 µg/ml respectively.

Macrofilaricidal activity

Mean time for paralysis (P in minutes) and mean time for death (D in minutes) were shown in Table 3. Among the four extracts, fixed oil and petroleum ether extract produced an activity which can be compared to that of the standard. Next comes the efficacy of ethanolic extract followed by aqueous extract. In general, the order of activity was produced by the various extracts was fixed oil > Petroleum ether extract > Ethanolic extract > Aqueous extract.

Table 1

Effect of various concentrations of petroleum ether, ethanolic, aqueous extract of leaves of *C. bonduc* against human lymphatic microfilariae, *W. bancrofti*

Extract	Concentration (µg/ml)	Numbers released	Numbers dead after 24 hrs	Mortality (%)	Corrected mortality using Abbot's formula (%)
Petroleum ether extract (Leaf)	25	10	2	20	26.6
		10	3	30	
		10	3	30	
	50	10	4	40	33.3
		10	3	30	
		10	3	30	
	100	10	6	60	60.0
		10	6	60	
		10	6	60	
Ethanolic extract (Leaf)	25	10	3	30	30.0
		10	3	30	
		10	3	30	
	50	10	5	50	53.3
		10	5	50	
		10	6	60	
	100	10	7	70	80.0
		10	9	90	
		10	8	80	
Aqueous extract (Leaf)	25	10	2	20	23.3
		10	2	20	
		10	3	30	
	50	10	3	30	26.6
		10	3	30	
		10	2	20	
	100	10	6	60	56.6
		10	5	50	
		10	6	60	

Table 2

Effect of various concentrations of fixed oil from seeds of
C.bonduc against human lymphatic microfilariae,
W. bancrofti

Extract	Concentration ($\mu\text{g/ml}$)	Numbers released	Numbers dead after 24 hrs	Mortality (%)	Corrected mortality using Abbot's formula (%)
Fixed oil (seed)	25	10	5	50	46.6
		10	4	40	
		10	5	50	
		10	6	60	
		10	7	70	
		10	7	70	
	100	10	7	70	80.0
		10	8	80	
		10	9	90	
		10	0	-	
		10	0	-	
		10	0	-	
Control	--	10	0	-	0.0

Table 3

Macrofilaricidal activity of various extracts of leaves and fixed oil from seeds of *C.bonduc* against *S.digitata*

Concentration (mg/ml)	25		50		100	
Test Solution	P	D	P	D	P	D
Standard (Albendazole)	34.16 ± 0.27	86.0 ± 0.30	21.3 ± 0.42	53.3 ± 0.50	17.0 ± 0.37	50.0 ± 0.42
Petroleum ether extract (Leaf)	208.3 ± 0.72	252.5 ± 1.39	171.8 ± 0.40	202.2 ± 0.61	151.7 ± 0.61	183.0 ± 0.51
Ethanolic extract (Leaf)	47.2 ± 0.40	113.0 ± 0.53	41.2 ± 0.47	95.8 ± 0.98	35.7 ± 0.33	81.5 ± 0.61
Aqueous extract (Leaf)	114.3 ± 0.55	142.6 ± 0.56	69.8 ± 0.65	106.3 ± 0.33	51.6 ± 0.20	92.8 ± 0.30
Fixed oil (Seed)	49.3 ± 0.49	109.8 ± 1.5	43.1 ± 0.40	79.6 ± 0.66	30.8 ± 0.30	60.0 ± 0.57

P= Paralysis in minutes, D=Death in minutes, n=6, the results given were Mean ± SEM. Test of significance between the mean parameters were performed using the analysis of variance (ANOVA) and the level of significance tested at P<0.01. In the control *S.digitata* lived upto 24 hrs.

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