

ANTHELMINTIC ACTIVITY OF VARIOUS
EXTRACTS OF LEAF AND FIXED OIL FROM THE
SEEDS OF *Caesalpinia bonduc* (L) ROXB

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Summary

The petroleum ether, ethanolic, aqueous extracts of leaf and fixed oil from the seeds of *Caesalpinia bonduc* (L) Roxb were screened for anthelmintic activity against *Toxocara canis* and *Eicenia fertida*. Fixed oil showed pronounced effect against *Toxocara canis* whereas petroleum ether extract exerted a pronounced effect against *Eicenia fertida*. In both the cases, the activity was dose dependent. The present study indicates that *Caesalpinia bonduc* (L) Roxb has significant anthelmintic activity. The maximal anthelmintic activity may be due to the presence of constituents like Hexadecadienoic acid, cassane diterpenes, furanoditerpene and furanoditerpenoids.

Key Words: *Caesalpinia bonduc*, anthelmintic activity, *Toxocara canis*, *Eicenia fertida*..

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

Introduction

Caesalpinia bonduc (L) Roxb (Family: Caesalpiniaceae) is a medicinally important, wild, thorny dicotyledon plant distributed in hotter parts, coastal areas, deltaic, eastern, western, southern parts of India and in other tropics, subtropics of the World^[1,2]. The ethno medical claim reveals that the plant is used in the treatment of helminthiasis, intestinal worms, arthritis, diabetes, eczema, boils, etc^[3, 4, 5]. Previously crude seed powder, its methanolic, aqueous extract were screened for its anthelmintic activity^[6] against *Neoscaris vitulorum* infected buffalo calves, anti ascarid activity^[7] in chickens, and in both the cases the crude seed powder was found to be potent. The present study aims at evaluating the anthelmintic activity (invitro) of the various extracts of leaf and fixed oil from the seeds of *Caesalpinia bonduc* (L) Roxb against *Toxocara canis* and *Eicenia fertida*.

Material and Methods

Plant Material Collection and Authentication

For this study the leaves and seeds of *C. bonduc* were collected during the month of May 2005, at Alagarkoil hills of Madurai district, Tamil Nadu, India. Care was taken to collect only the healthy leaves and seeds for the preparation of leaf extract and isolation of oil respectively. The leaves and seeds were authenticated at the Department of Botany, The American College, Madurai and voucher specimens (PCG14CbL & PCG14CbS) has been deposited in Department of Pharmacognosy, Madurai Medical College, Madurai.

Preparation of Extracts

Petroleum ether, ethanolic, aqueous extract of leaf

The collected leaves were shade dried, powdered and extracted with petroleum ether (60° - 80°C), ethanol

individually by cold maceration process. The aqueous extract was prepared by refluxing the shade dried, powdered leaves with distilled water. The crude extracts were evaporated to dryness and the residues (petroleum ether extract – 6.85 % w/w, ethanolic extract – 3.97 % w/w, aqueous extract – 7.15 % w/w) were maintained at refrigerator until used.

Extraction of oil from seed

The decorticated seeds were packed in soxhlet apparatus and extracted with petroleum ether (60° - 80°C) for 6 hrs. Then the solvent was evaporated to yield a pale yellow, viscous oil with disagreeable odour (21.7 %) and the oil was stored at room temperature until used.

Preparation of test samples

Test samples for the anthelmintic study were prepared by dissolving the dried extracts in 1% gum acacia in normal saline.

Worm collection and authentication

Toxocara canis were collected from dogs, authenticated by the Assistant veterinary surgeon, Madurai Medical College, Madurai and *Eicenia fertida* (earth worm) were collected from the moist soil, inside the campus of Madurai Medical College, authenticated at the Department of Zoology, The American College, Madurai.

Experimentation

The anthelmintic activity of the various extracts of leaf and fixed oil from the seeds of *C. bonduc* was determined by using the method of Ajaiyeoba et al ^[8]. Two worms of same type were placed in 9 cm petridishes with test samples in three different concentrations (25, 50 and 100 mg/ml) respectively. This was done in triplicate for both the types of worms.

When movement of any kind could not be observed, except when the worms were shaken vigorously, mean times for paralysis (P in minutes) were taken. Times of death of worms (D in minutes) were recorded after confirming that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Albendazole at a concentration similar to that of the test samples were used as standard compound, while normal saline was used as control.

Results

The time taken for paralysis (P) and death (D) for both the type of worms were captured in Table 1 and 2. In case of *T. canis*, among the four test samples, the activity exerted by fixed oil was pronounced at the concentration of 100 mg/ml, whereas the activity exerted by the petroleum ether, ethanolic, aqueous extract were comparable to that of the standard. The decreasing order of activity was exerted by fixed oil, petroleum ether extract, ethanolic extract and aqueous extract. In case of *E. fertida*, among the four test samples, the activity exerted by the petroleum ether extract and ethanolic extract at 100mg/ml concentration were prominent, where as the effect of fixed oil, aqueous extract were comparable to that of the standard. The decreasing order of activity was exerted by petroleum ether extract, ethanolic extract, fixed oil and aqueous extract.

Discussion

The anthelmintic activity of the extracts of leaf and fixed oil from the seeds of *C. bonduc* may be due to the presence of constituents like Hexadecadienoic acid ^[9], cassane diterpenes ^[10], cassane furanoditerpene ^[11] and cassane furanoditerpenoids ^[12]. However, the activity exerted by the different extracts varies with the types of worms used, in general the fixed oil, petroleum ether, ethanolic extract showed a pronounced activity. Hence a detailed study should be taken up for the isolation and characterization of the compounds responsible for the anthelmintic activity.

Table 1. Anthelmintic activity of various extracts of leaf and fixed oil from the seeds of *C. bonduc* against *T. canis*

Concentration mg/ml	25		50		100	
Test Solution	P	D	P	D	P	D
Standard (Albendazole)	39 ± 0.54	89.3 ± 0.42	23 ± 0.36	63 ± 0.36	15.3 ± 1.16	52 ± 0.31
Petroleum ether extract (leaf)	44.83 ± 0.31	95 ± 0.36	35.3 ± 0.42	75.3 ± 0.21	24.8 ± 0.48	67.5 ± 0.99
Ethanolic extract (leaf)	46.5 ± 0.57	98.8 ± 0.55	40 ± 0.78	81.6 ± 0.56	30.6 ± 0.34	71 ± 0.58
Aqueous extract (leaf)	53 ± 0.36	112 ± 1.04	42.2 ± 0.75	90.2 ± 0.48	33.8 ± 0.48	75.8 ± 0.31
Fixed oil (seeds)	40.81 ± 0.31	88 ± 0.36	31 ± 0.36	70 ± 0.36	23.2 ± 0.40	62.8 ± 0.40

P = Paralysis in minutes, D = Death in minutes, n = 6, the results given were Mean ± SD. 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 *T. canis* lived up to 48 hrs.

Table 2. Anthelmintic activity of various extracts of leaf and fixed oil from the seeds of *C. bonduc* against *E. fertida*

Concentration (mg/ml)	25		50		100	
	P	D	P	D	P	D
Standard (Albendazole)	12.3 ± 0.21	19.5 ± 0.22	9.5 ± 0.22	15.6 ± 0.21	7.3 ± 0.21	12.5 ± 0.22
Petroleum ether extract (leaf)	15.0 ± 0.26	18.3 ± 0.21	12.3 ± 0.34	14.5 ± 0.35	8.5 ± 0.22	11.3 ± 0.21
Ethanollic extract (leaf)	16.0 ± 0	23.3 ± 0.21	12.5 ± 0.22	17.3 ± 0.21	8.5 ± 0.22	14.8 ± 0.40
Aqueous extract (leaf)	22.8 ± 0.40	28.3 ± 0.21	19.0 ± 0.25	21.3 ± 0.34	14.5 ± 0.35	23.6 ± 0.22
Fixed oil (seed)	23.6 ± 0.22	26.8 ± 0.34	16.3 ± 0.22	18.6 ± 0.33	10.6 ± 0.33	13.0 ± 0.36

P = Paralysis in minutes, D = Death in minutes, n = 6, the results given were Mean ± SD. 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 *E. fertida* lived up to 48 hrs.

References

1. Anonymous, Wealth of India, Vol. II, CSIR Publications, Delhi. , 1956 pp. 6 - 8.
2. Gamble JS. Flora of Presidency of Madras. Botanical Survey of India. Vol. II, Calcutta. 1967, pp. 278 - 279.
3. Nadkarni KM. Indian Materia Medica. 3rd edition, Vol I, Popular Book Depot, India. 1954, pp. 229.
4. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal plants. CSIR, New Delhi. 1956, pp. 43 - 44.
5. Warriar PS, (Vaidyaratnam). Indian Medicinal Plants. Vol I, Arya Vaidya Sala, Kottakkal, Orient Longmann Limited, India. 1996, pp. 320 - 21.
6. Akhtar MS, Javed I, Hayat CS, Shah B. Efficacy and safety of *Caesalpinia crista* Linn. seeds: its extracts in water and methanol against natural *Neoascaris vitulorum* infection in buffalo-calves. Pakistan Veterinary Journal. 1985, 5(4), 192 - 196.
7. Javed I, Akhtar MS, Rahman ZU, Khaliq T, Ahmad M. Comparative anthelmintic efficacy and safety of *Caesalpinia crista* seed and piperazine adipate in chickens with artificially induced *Ascaridia galli* infection. Acta Vet Hung. 1994, 42(1): 103-109.
8. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In vitro anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extracts. Pharmaceutical Biology. 2001, 39 (3), 217 – 220(4).
9. Shameel S, Usmanghani K, Ali MS, Ahmad VU. *Caesalpinia bonduc* (L.) Roxb. seed oil: lipid composition assessment. Pak J Pharm Sci. 1997, 10 (1): 29 - 38.
10. Kinoshita T. Chemical studies on the Philippine crude drug calumbibit (seeds of *Caesalpinia bonduc*): the isolation of new cassane diterpenes fused with alpha,beta-butenolide. Chem Pharm Bull (Tokyo). 2000, 48 (9): 1375 - 1377.

11. Jadhav AN, Kaur N, Bhutani KK. A new furanoditerpenoid marker for the distinction between the seeds of two species of *Caesalpinia*. *Phytochem Anal.* 2003, 14 (5): 315 - 318.
12. Pudhom K, Sommit D, Suwankitti N, Petsom A. Cassane furanoditerpenoids from the seed kernels of *Caesalpinia bonduc* from Thailand. *J Nat Prod.* 2007, 70 (9): 1542 - 1544.

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