

**PHYTOCHEMICAL INVESTIGATION AND INVITRO ANTHELMINTIC ACTIVITY OF
BAUHINIA RACEMOSA LINN (LEGUMINACEAE)**

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Summary

Stem bark, leaves, seeds and root of *Bauhinia Racemosa* Linn (Leguminaceae) were separately dried, powdered and extracted with methanol in Soxhlet extractor. Anthelmintic activity of these extracts was evaluated on Indian adult earthworms, *Pherentima posthuma*. Results showed that the leaves of *B. Racemosa* took less time to cause paralysis and death of the earthworms; therefore, leaves were extracted successively with petroleum ether, chloroform, ethyl acetate and methanol in Soxhlet extractor. Each extract was studied for their anthelmintic activity, which involved determination of the time of paralysis and time of death of the worms. Results showed that the petroleum ether extract of leaves of *B. Racemosa* at 60 mg/ml was most potent as compared to other extracts and standard drug albendazole. Order of potency was observed as petroleum ether > ethyl acetate > methanol > chloroform extract. It can be concluded that anthelmintic activity of the leaves of *B. Racemosa* is due to the active principles present in the petroleum ether and ethyl acetate extracts. The purpose of this project was to evaluate invitro anthelmintic activity of various extract of *Bauhinia Racemosa* Linn (Leguminaceae). Albendazole (20 mg/ml) and distilled water were included in the assay as standard drug and control, respectively.

Keywords: *Bauhinia Racemosa*, *Pheritima posthuma*, In-vitro anthelmintic activity, Leguminaceae.

Introduction

The plant *Bauhinia racemosa* Lam. belongs to the Caesalpiniaceae Family. It is popularly known as Sittacha (Tamil) and occurs frequently in India, Ceylon, China and Timor. The stem bark of the plant is an astringent and is used in the treatment of headache, fever, skin diseases, tumors, diseases of the blood, dysentery and diarrhea.^[1] β -sitosterol and β -amyrin probably responsible for the related popular use, were isolated from the stem bark of this plant.^[2] Beside these compounds, at least five flavonols (Kaempferol and Quercetin) and two coumarin (scopoletin and scopolin) were also isolated from the leaf.^[3] Stilbene (Resveratrol) was isolated from the heartwood of *B. racemosa*.^[4] Pharmacological studies of the plant revealed that the ethanol extract of leaves of *B. racemosa* shows analgesic, antipyretic, anti-inflammatory and antispasmodic,^[5] and antimicrobial activity.^[6] The fresh flower buds of the plant showed antiulcer activity.^[7] The cytotoxicity against CA-9 KB in cell culture, hypotensive and hypothermic activities were reported from the hydroalcoholic extract of *B. racemosa*.^[8]

Material and Methods

Plant material

Stem bark, leaves, seeds and root of *Bauhinia Racemosa* Linn (Leguminaceae) were collected from Ahmednagar district, Maharashtra (India) in August 2007. The plant specimen was authenticated from Botanical Survey of India, Pune (Voucher specimen no. BRLCG1).

Animals

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.^[9, 10]

Drugs and chemicals

Albendazole (Pfizer Ltd., Bangalore), Petroleum ether (PCL, Pune), Chloroform (PCL, Pune), ethyl acetate (PCL, Pune), Methanol A. R. (PCL, Pune), DMF (PCL, Pune), Saline water (Nurilife, Ahmedabad).

Preparation of extracts

Dried and coarsely powdered stem bark, leaves, seeds and roots of *B. Racemosa* (500 g, each) was separately subjected to extraction in Soxhlet extractor using methanol as a solvent. The extracts of stem bark, leaves, seeds and roots of *B. Racemosa* were concentrated by vacuum distillation and then dried in open air to yield 6.2%, 6.3%, 5.2%, 6.0% of extracts, respectively. The most active part i.e. leaves (1000 g) (based on primary anthelmintic screening) were dried, coarsely powdered and subjected to successive solvent extraction in Soxhlet extractor by using petroleum ether (60-80°C), chloroform, ethyl acetate and methanol as a solvent. The respective extracts were concentrated by vacuum distillation and then dried in open air to give 6.7%, 4.5%, 7.9% and 6.0% of extracts, respectively.^[11]

Anthelmintic Activity^[12, 13]

All the extracts of stem bark, leaves, seeds and roots of *B. Racemosa* were dissolved in minimum amount of DMF and then volume is adjusted to 10 ml with saline water. All extracts and albendazole solutions were freshly prepared before starting the experiment. Six groups, of six earthworms each were released into 10 ml of desired formulations as follows; vehicles (5% DMF in normal saline), Albendazole (20 mg/ml), or total methanolic extracts of stem bark, leaves, seeds and roots of *B. Racemosa* (20 mg/ml, each) in normal saline containing 5% DMF. In the second set of experiment, fourteen groups of six earthworms were released in to 10 ml of desired formulations as follows; vehicle (5% DMF in normal saline), albendazole (20 mg/ml), or petroleum ether extract or chloroform extract or ethyl acetate extract or methanolic extract of leaves of *B. Racemosa* (20 mg/ml, 40 mg/ml, 60 mg/ml each) in normal saline containing 5% DMF. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). Death was concluded when the worms lost their motility followed with fading away of their body colors. All the results were expressed as a mean \pm SEM of six animals in each group.

Results and Discussion

The data revealed that total methanolic extracts of various parts of the plant *B. Racemosa* showed significant anthelmintic activity at 20 mg/ml concentrations, amongst all, total methanolic extract of leaves of *B. Racemosa* showed best anthelmintic activity as shown in Table 1. Results are comparable with standard drugs Albendazole, at same concentration. In order to find out active constituents from leaves, which are responsible for the activity whether polar or nonpolar compounds, successive extraction of leaves of *B. Racemosa* was done by using solvents like petroleum ether, chloroform, ethyl acetate and methanol. All extracts were screened for anthelmintic activity. The results show that petroleum ether extract (at 60 mg/ml) of leaves of *B. Racemosa* took the least time to cause paralysis (**2.11min**) and death (**2.02 min**) of the earthworms, followed by ethyl acetate, methanolic extracts and chloroform extract respectively. The standard drug, Albendazole shows paralysis at **2.06** min and death after 1.05 min (at 60 mg/ml).

Table 1. Anthelmintic activity of methanolic extracts of stem bark, leaves, seeds and root of *Bauhinia Racemosa* Linn.

Treatment	Dose mg/ml	Time of paralysis (min) \pm SEM	Time of Death (min) \pm SEM
BME	20	8.48 \pm 0.00577	10.53 \pm 0.0115
LME	20	3.45 \pm 0.0023	3.03 \pm 0.065
SME	20	9.65 \pm 0.01202	8.19 \pm 0.0318
RME	20	8.33 \pm 0.00881	7.52 \pm 0.087
ALBENDAZOLE	20	3.08 \pm 0.00577	2.23 \pm 0.1453
CONTROL	5% DMF in normal saline	-	-

BME- total methanol extract of bark, LME- total methanol extract of leaves, SME- total methanol extract of seeds, RME- total methanol extract of roots. All the results were expressed as a mean \pm SEM of six animals in each group.

Table 2. Anthelmintic activity of various extracts of leaves of *Bauhinia Racemosa* Linn.

Treatment	Dose mg/ml	Time of paralysis (min) \pm SEM	Time of Death (min) \pm SEM
PEL	20	3.57 \pm 0.0094	2.53 \pm 0.45
	40	3.45 \pm 0.00881	2.45 \pm 0.091
	60	2.11 \pm 0.056	2.02 \pm 0.095
CHL	20	9.48 \pm 0.0057	5.03 \pm 0.015
	40	8.34 \pm 0.012	4.61 \pm 0.011
	60	7.29 \pm 0.0133	3.2 \pm 0.0077
EAL	20	4.65 \pm 0.012	3.196 \pm 0.038
	40	3.58 \pm 0.0078	2.363 \pm 0.033
	60	3.48 \pm 0.0081	1.38 \pm 0.033
MEL	20	8.33 \pm 0.0081	4.52 \pm 0.0081
	40	7.24 \pm 0.088	3.19 \pm 0.034
	60	7.25 \pm 0.0020	3.01 \pm 0.0037
ALBENDAZOLE	20	3.08 \pm 0.0057	2.23 \pm 0.14
	40	2.07 \pm 0.0015	1.24 \pm 0.0088
	60	2.06 \pm 0.0059	1.05 \pm 0.0057
CONTROL	5% DMF in normal saline	-	-

PEL- petroleum ether extract of leaves, CHL- chloroform extract of leaves, EAL- ethyl acetate extract of leaves, MEL- methanol extract of leaves. All the results were expressed as a mean \pm SEM of six animals in each group.

Results of preliminary phytochemical tests suggest that petroleum ether extract of leaves contain sterols, carbohydrates, triterpenes and fatty substances; chloroform extract contain sterols; ethyl acetate extract of leaves contain flavanoids, glycosides and methanolic extract of seeds contain tannins, glycosides and alkaloids. It can be concluded that active constituents responsible for anthelmintic activity are present in the petroleum ether and ethyl acetate extracts of leaves of *B. Racemosa*. This indicates that the anthelmintic

principles are nonpolar compounds. The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the faeces of human being and animals. The extracts not only demonstrated this property, they also caused death of the worms. And further study was carried out for isolation of the chemical constituent responsible for anthelmintic activity.

Further studies using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of leaves of *B. Racemosa* as an anthelmintic drug. The drug may be further explored for its phytochemical profile to identify the active constituent responsible for anthelmintic activity.

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