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Research Article

IN VITRO ANTHELMINTIC ACTIVITY OF BAUHINIA VARIEGATA BARK (LEGUMINOSAE)

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ABSTRACT

Aqueous and Chloroform extract of bark of *Bauhinia Variegata* were investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*. All extracts exhibited a dose dependent (25, 50 and 100 mg/ml) inhibition of spontaneous motility (paralysis) and time of death of the worms. Extract obtained from bark not only killed the *Pheretima posthuma* but also killed the *Ascardia galli*. The observations were comparable with standard drug Piperazine citrate at a concentration of 20 mg/ml and distilled water as control. Maximum vermicide activity was shown by both extract at the concentration of 100mg/ml. From the experiment performed, it can be said that the aqueous and chloroform extract of bark of *Bauhinia variegata* bearing a potential anthelmintic activity.

Keywords: Anthelmintic activity, *Ascardia galli, Bauhinia Variegata*, *Pheretima posthuma,* Piperazine citrate.

INTRODUCTION

Helminthes are the most common infections in man, affecting a large proportion of the world's population. Parasitic diseases may cause severe morbidities including lymphatic filarisis (cause of elephantiasis), onchocerciasis and schistosomiasis.[1-2] Development of resistance to most of commercially available anthelmintic became a severe problem worldwide. [3]

Bauhinia variegata Linn. (Leguminosae), commonly known as "Kachnar" is an herbaceous plant, reaching up to 6-12 meters. The leaves are shaped a little like cow's hoof and white to pinkish flowers. The flowers are hermaphrodite (have both male and female organs). Petals pale purple or rose or white or yellow, obovate 4-6 cm long, 2-3 cm broad. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils. The plant prefers acid, neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or no shade. It requires moist soil.[4-5] *Bauhinia Variegata* widely distributed plant for the Leprosy, Menorrhagia, Impurites of blood, Tuberculous gland, wound, ulcers and asthma etc.[6-7]*Bauhinia Variegata* is an indigenous medicinal plant with pharmacological properties similar to rasayanas.[8]The bark powder of the plant is a major ingredient of the herbal tonic Kanchanar guggul, an ayurvedic remedy prescribed to increase the white blood cells. Phytochemical characterization shows the presence of tannins, steroids, alkaloids, flavonoids and saponin in the stem bark of *Bauhinia Variegata* Linn.[9]The ethanolic extract of stem bark of *Bauhinia Variegata* Linn. contains β sitosterol, lupeol, vitamin C, kaempferol, flavanone and quercetin. Some studies have reported its antitumor, anti-ulcer antibacterial and antifungal activites.[10-13]

MATERIALS AND METHOD

Plant materials

The fresh bark of *Bauhinia Variegata* were collected from local area of sonai, Maharashtra, India and identified and authenticated from Botanical survey of India, Pune the bark was air dried and powdered with a mechanical grinder, filtered and fine powder was stored in a non-toxic polyethylene bag.

Drug and chemicals

The following drugs and chemicals were used:

Piperazine citrate (Pankaj Medicos, India), Tween-80 (Acme chemicals, India), Chloroform (Research Lab Fine chem. Industries, India)

Preparation of the extract [14]

Aqueous Extract (Maceration method)

Air-dried fine bark powdered material (200 gm) of *Bauhinia Variegata* was kept for maceration with 1000 ml of distilled water for 12 hr. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be 3.65 percent.

Chloroform Extract (Continuous Soxhlet extraction method)

The powder was first defatted with petroleum ether and then soxhlet-extracted with Chloroform which is further evaporated to dryness to obtain chloroform extract.

Preliminary Phytochemical screening [15-17]

The extract was subjected to phytochemical screening and the preliminary chemical examination of Chloroform extract revealed the presence of steroids, flavonoids, tannins, coumarins, carbohydrates and reducing sugars. Flavonoids exhibit varied biological activities that include analgesic, anti-inflammatory, antioxidant, hepatoprotective and antiulcer activities. Tannins are protectants. Based on this, it was contemplated to carry out the screening of chloroform extract for analgesic, anti-inflammatory activities.

Anthelmintic Bioassay

The aqueous and chloroform extracts of bark of *Bauhinia Variegata* were investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*. Different concentrations of the extract were tested in the bioassay, which involved determination of the time of paralysis and the time of death of the worms. Piperazine citrate was included as standard reference and distilled water as control.

Anthelmintic assay was performed on healthy adult Indian earthworm, *Pheretima posthuma* due to its anatomical resemblance with the intestinal roundworm parasite of human beings.[18-22]

Because of the easy availability, earthworms have been used widely for the Preliminary evaluation of the anthelmintic activity in vitro. Indian adult earthworm (*Pheretima posthuma*) were collected from Mahatma Phule Krushi vidyapeeth, Rahuri, Maharashtra, and washed with normal saline to remove all faecal matter and used for the anthelmintic activity. The earthworm of 4-6 cm in length and 0.1-0.2 cm in width were used. *Ascardia galli* worms are easily available in plenty from freshly slaughtered fowls and they were used for the anthelmintic activity. [23-27]

Activity against earthworms

The anthelmintic assay was carries as per the method of Ajaiyeoba et al. with minor modifications.[28]Suspension of aqueous extract and chloroform extract of *Bauhinia Variegata* at different concentration

(25, 50 and 100 mg/ml) were prepared by using Tween-80 (0.1%) as a suspending agent and final volume were made to 50 ml for respective concentration. Piperazine citrate 20mg/ml was prepared by using Tween-80 (0.1%) as a suspending agent and used as reference.[29]

Six worms about the same size were released in 50 ml of sample with desired concentration. They were observed for their spontaneous motility and evoked responses. The paralytic score was recorded at different time intervals. Immediately after inhibition of response to external stimuli, the worms were placed in fresh water and observed for recovery. Duration required for final recovery/death was noted; mean paralytic score was plotted against time.[30]

The death and/or total paralysis time was recorded at room temperature. The death of the worm was ascertained by transferring it into a beaker containing hot water (50^0C), which stimulated and induced movements if the worm was alive. Same experiment was done for *Ascardia galli* worms.

Table 1: Anthelmintic activity of aqueous and chloroform extract of *Bauhinia variegata*

Plant Extract	Concentration (mg/ml)	*Pheretima posthuma*		*Ascardia galli*	
		Paralysis (min)	Death (min)	Paralysis (min)	Death (min)
AE	25	74.4 + 0.43	85.3 + 0.93	75.8 + 0.42	84.1 + 1.00
	50	51.5 + 0.55	66.1 + 1.05	53.1 + 0.77	65.2 + 0.61
	100	36.6 + 0.65	49.8 + 0.57	36.9 + 0.43	50.3 + 1.24
CE	25	70.5 + 0.83	75.4 + 0.38	73.6 + 1.71	79.0 + 1.93
	50	36.4 + 0.53	40.5 + 0.47	50.9 + 2.11	63.1 + 1.51
	100	24.8 + 0.51	27.1 + 0.28	29.6 + 1.93	31.6 + 2.10
PC	20	21.9 + 1.71	24.0 + 0.91	23.7 + 1.32	27.0 + 0.48
Control	-	-		-	

AE-Aqueous extract, CE- Chloroform Extract, All the values expressed Mean + SD; n=6 in each group.

RESULT AND DISCUSSION

Preliminary phytochemical screening of chloroform extract revealed the presence of steroids, flavonoids, tannins, coumarins, carbohydrates and reducing sugars like phytoconstitutents (Table1) may be responsible to show a potent anthelmintic activity. From the observation made the extracts of plant of *Bauhinia variegata* was found to show a potent anthelmintic activity when compared to standard drug.

Aqueous extract of *Bauhinia variegata* (25 mg/ml) showed the time of paralysis and death 74.4 min and 85.31 min respectively against the *Pheretima posthuma*, whereas against the *Ascardia galli* it showed 75.8 min and 84.1 min time for paralysis and death respectively. For 50 mg/ml of concentration the time of paralysis and death was found to be 51.53 min and 66.13 min respectively against the *Pheretima posthuma*, whereas against the *Ascardia galli* it showed 53.1 min and 65.2 min for paralysis and death respectively. For 100 mg/ml of concentration the time of paralysis and death was found to be 36.6 min and 49.8 min respectively against the *Pheretima posthuma*, whereas against the *Ascardia galli* it showed 36.9 min and 50.3 min respectively.

For the chloroform extract of *Bauhinia variegata* (25 mg/ml) showed the time of paralysis and death 70.5 min and 75.4 min respectively against the *Pheretima posthuma*, whereas against the *Ascardia galli* it showed 73.6 min and 79 min time for paralysis and death respectively. For 50 mg/ml of concentration the time of paralysis and death was found to be 36.4 min and 40.5 min respectively against the *Pheretima posthuma*, whereas against the *Ascardia galli* it showed 50.4 min and 63.1 min for paralysis and death respectively. For 100 mg/ml of concentration the time of paralysis and death was found to be 24.8 min and 27.1 min respectively against the *Pheretima posthuma*, whereas against the *Ascardia galli* it showed 29.6 min and 34.6 min respectively.

The observation with Piperazine citrate showed the time of paralysis and death 21.9 min and 24 min respectively against the *Pheretima posthuma*, whereas against the *Ascardia galli* it showed 23.7 min and 27 min respectively. For 20 mg/ml concentration, it observed that both the extract showed a remarkable anthelmintic potential against the *Pheretima posthuma* and *Ascardia galli.* The anthelmintic activity of *Bauhinia variegata* extract due to the presence of active constitutents i.e. steroid, flavonoids, tannins etc. Further, there is scope to evaluate the active principals of *Bauhinia variegata* for their anthelmintic activity to open the new era for natural anthelmintic.

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