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## *In vitro* anthelmintic activity of *Barleria buxifolia* on Indian adult earthworms and estimation of total flavonoid content

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## PEER REVIEW

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**Comments**

This is a good study in which the authors evaluated the leaf extract for the anthelmintic activity. The results were promising and good which will help researchers for the further review. Details on Page S235

## ABSTRACT

**Objective:** To study the anthelmintic activity of *Barleria buxifolia* leaf and to estimate the total flavonoid content.

**Methods:** The aqueous and ethanolic leaf extracts were prepared and these were analyzed for total flavonoid content by aluminium chloride colorimetric method and *Pheretima posthuma* was used for anthelmintic activity by using the different concentrations (10, 20, 40, 80 and 100 mg/mL).

**Results:** All the investigational extracts showed an anthelmintic activity at concentration of 10 mg/mL. The ethanolic extract of 100 mg/mL has produced a significant effect ( $P < 0.001$ ) when compared to aqueous extract. The total flavonoid content was found to be 5.67 mg QE/100 g.

**Conclusions:** From the above study, the leaf extract has shown a good anthelmintic activity.

## KEYWORDS

Helminthiasis, *Barleria buxifolia*, Aluminium chloride colorimetric method, *Pheretima posthuma*

### 1. Introduction

Helminthic diseases have worldwide distribution. They effect billions of people in endemic areas and can result in serious complications. According to World Health Organization statistics, nearly more than two billion people suffer from parasitic worm infections<sup>[1]</sup>. Helminthiasis is a disease in which a part of the body is infested with worms, such as pinworms, round worms and tapeworms. Typically, the worms reside in gastro intestinal tract, but may also burrow into liver and other organs. Infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation<sup>[2]</sup>. Anthelmintics are drugs that either kill or expel parasitic worms. The majority of drugs available to treat these infections possess some common side effects such as nausea, vomiting, abdominal pain, expulsion of ascaris from mouth or nose,

allergic reactions, loss of hair, urticaria, granulocytopenia, fall in blood pressure, sedation, fever, and body ache<sup>[3]</sup>. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug.

*Barleria buxifolia* Linn. (*B. buxifolia*), belonging to the family Acanthaceae, is an erect under shrub with long spines and white to pink flower and used as an ornamental hedge in the garden. The roots and leaves were used traditionally in cough, bronchitis, inflammation (applied to swellings)<sup>[4]</sup>.

### 2. Materials and methods

#### 2.1. Plant material

The leaves of *B. buxifolia* were procured from the forests and were authenticated.

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## 2.2. Preparation of extract

Leaves of *B. buxifolia* were dried in shade and coarsely powdered. The powdered leaves were subjected to Soxhlet extraction by water and ethanol used as a solvents for 72 h. The extracts were concentrated by distilling off the solvent and then evaporating to dryness on the water bath.

## 2.3. Drugs and chemicals

Albendazole (Alkem Laboratories Ltd.) and all the chemicals used in the study were of analytical grade.

## 2.4. Preliminary phytochemical screening

Phytochemical screening of aqueous and ethanolic extracts were carried out to evaluate the presence of various active constituents.

## 2.5. Estimation of total flavonoid content

Total flavonoid content was measured by the aluminium chloride colorimetric assay[5]. An aliquot (1 mL) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 µg/mL) was added to a 10 mL volumetric flask containing 4 mL of distilled water. To the flask, 0.3 mL of 5% NaNO<sub>2</sub> was added and after 5 min, 0.3 mL of 10% AlCl<sub>3</sub> was added. After 5 min, 2 mL of 1 mol/L NaOH was added and the volume was made upto 10 mL with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

## 2.6. Experimental model

Adult Indian earth worms, *Pheretima posthuma* (*P. posthuma*) having anatomical and physiological resemblance with intestinal roundworm parasite of the human being were used to evaluate anthelmintic activity[6,7]. These were collected from moist soil and washed with normal saline to remove all fecal matter.

## 2.7. Anthelmintic activity

Indian adult earth worms 4–5 cm in length and 0.1–0.2 cm in width were used for the *in vitro* anthelmintic bioassay of aqueous extract. The worms were divided into the respective groups containing six earthworms in each group. The aqueous and ethanolic extracts were prepared at different concentrations (10, 20, 40, 80, and 100 mg/mL), the standard drug albendazole (10 mg/mL) in 50 mL of water, and the earth worms were put in the solutions and observed for anthelmintic activity. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color. Six worms of about the same size per Petri dish were used. They were observed for their

spontaneous motility and evoked responses. Observations were made for the time taken to paralysis and death of individual worm[8].

## 2.8. Statistical analysis

All results are expressed as mean±SD, and groups of data were compared with analysis of variance (ANOVA) followed by Dunnett's test. Values would be considered statistically significant when *P* value was less than 0.001.

## 3. Results

### 3.1. Preliminary phytochemical screening

Results from the phytochemical screening revealed the presence of alkaloids, flavonoids, steroids and tannins in both extracts.

### 3.2. Estimation of total flavonoid content

The flavonoid contents were present in aqueous and ethanolic extracts of *B. buxifolia* leaves, and the total flavonoid content was found to be 5.67 mg QE/100 g.

### 3.3. Anthelmintic activity

The crude aqueous extract has produced a dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. The dose of 100 mg/mL has shown the significance (*P*<0.001) when compared to albendazole (10 mg/mL). The ethanolic extract has taken the time of (37.75±2.06) min for paralysis and time of death was (89.00±1.82) min, and the aqueous extract has taken the time of (64.00±2.16) min for paralysis and time of death was (150.50±2.64) min. The results showed that the ethanolic extract showed better activity than that of aqueous extract (Table 1).

**Table 1**

Anthelmintic activity of *B. buxifolia* on *P. posthuma*.

Groups	Drug treatment	concentration (mg/mL)	Time for paralysis (min)	Time for death (min)
I	Albendazole	10	33.75±3.50	44.55±2.50
II	AE	10	86.75±5.78**	185.25±5.25***
III	AE	20	81.75±3.30**	175.00±2.58***
IV	AE	40	73.75±2.62**	164.25±3.30***
V	AE	80	71.50±2.88***	158.25±2.06***
VI	AE	100	64.00±2.16***	150.50±2.64***
VII	EE	10	53.00±2.58**	103.00±2.16***
VIII	EE	20	51.00±2.16**	100.25±1.70***
IX	EE	40	46.00±1.82***	96.50±1.29***
X	EE	80	43.75±2.06***	93.75±1.50***
XI	EE	100	37.75±2.06***	89.00±1.82***

Values are expressed as mean±SD. The results were analysed by analysis of variance (ANOVA) followed by Dunnett's *t*-test. *P*<0.001 when compared with Group II. AE: Aqueous extract; EE: Ethanolic extract.

## 4. Discussion

The assay of biological activity, aqueous and ethanolic extract used to evaluate anthelmintic activity has shown the dose dependent activity. The mean±SD values were calculated for each extract. The result of anthelmintic activity on earthworm *P. posthuma* reveals that the different concentrations used for aqueous and ethanolic extracts have shown paralysis and death of earthworms, which were compared with albendazole as reference drug. Albendazole acts by inhibiting the polymerization of helminth  $\beta$ -tubulin, and thus interfering with microtubule dependent functions like glucose uptake<sup>[3]</sup>.

From phytochemical screening, the aqueous and ethanolic leaf extracts showed the presence of alkaloids, flavonoids, tannins and steroids which have been associated with anthelmintic activity<sup>[9]</sup>. The alkaloids and tannins present in the leaf are responsible for the anthelmintic activity. Possible mechanism of anthelmintic activity of leaf extract may be due to tannins which bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death<sup>[10]</sup>. The second possible mechanism may be the alkaloids which act on central nervous system and cause paralysis of the *P. posthuma* worms<sup>[11]</sup>.

From the above study, we conclude that the aqueous and ethanolic extracts of *B. buxifolia* have shown a good anthelmintic activity, and further *in vivo* studies need to be conducted to evaluate the effectiveness of the extracts which can be used as anthelmintic drug.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### Background

As the helminthic infections pose an great economic burden in the developing countries by using synthetic drugs which cause number of side effects. This creates an intention in researchers for the search of novel therapeutics to eradicate the infections.

### Research frontiers

Studies were conducted to evaluate the anthelmintic activity on earthworms which serve as the best screening models for anthelmintic drugs. *P. posthuma*, an Indian

earthworm, was used in the study.

### Related reports

The present data about the anthelmintic activity of *B. buxifolia* leaf was conducted according to methods adopted in recent articles. Total flavonoid content was estimated and has shown good results.

### Innovations & breakthroughs

The data suggest that with the use of plant based drugs, there will be less number of side effect profile.

### Applications

From this study, it can be concluded that the leaf extract can be used as an alternative to synthetic drugs for the treatment of helminthiasis.

### Peer review

This is a good study in which the authors evaluated the leaf extract for the anthelmintic activity. The results were promising and good which will help researchers for the further review.

## References

- [1] Danquah CA, Koffur GA, Annan K, Ketor EC. The anthelmintic activity of *Vernonia amygdaline* (Asteraceae) and *Alstonia boonnia* De wild (Apocynaceae). *J Med Biomed Sci* 2012; **1**(1): 21–27.
- [2] Idika IK, Okonkwo EA, Onah DN, Ezeh IO, Iheagwam CN, Nwosu CO. Efficacy of levamisole and ivermectin in the control of bovine parasitic gastroenteritis in the sub-humid savanna zone of southeastern Nigeria. *Parasitol Res* 2012; **111**(4): 1683–1687.
- [3] Tripathi KD. *Essentials of medical pharmacology*. 6th ed. New Delhi: Jaypee Brothers Medical Publishers Ltd.; 2008, p. 759–766.
- [4] Chetty KM, Sivaji K, Rao KT. *Flowering plants of Chittoor district*. Tirupathi: Students Offset Printers; 2008, p. 253.
- [5] Jia ZS, Tang MC, Wu JM. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 1999; **64**: 555–559.
- [6] Pillai LS, Nair BR. A comparative study of the anthelmintic potential of *Cleome viscosa* L. and *Cleome burmanni* W. and A. *Indian J Pharm Sci* 2011; **73**(1): 98–100.
- [7] Ong HC, Nordiana M. Malay ethno-medicobotany in Machang, Kelantan, Malaysia. *Fitoterapia* 1999; **70**: 502–513.
- [8] Ajaiyeoba EO, Onocha PA, Olarenwaju OT. *In vitro* anthelmintic properties of *Buchholzia coriacea* and *Gynandropsis gynandra* extract. *Pharm Biol* 2001; **39**: 217–220.
- [9] Sarojini N, Manjari SA, Kanti CC. Phytochemical screening and anthelmintic activity study of *Saraca indica* leaves extracts. *Int Res J Pharm* 2011; **2**(5): 194–197.
- [10] Hussain A, Sonari AK, Md. Ahmed P, Wahab S. *In vitro* anthelmintic activity of *Coleus aromaticus* root in Indian adult earthworm. *Asian Pac J Trop Dis* 2012; **2**(Suppl 1): S425–S427.
- [11] Suman A, Kumar DG, Kumar BD, Raj CR. Preliminary phytochemical investigation and anthelmintic activity of *Acacia suma* (Roxb) bark. *Int Res J Pharm* 2011; **2**(1): 136–141.