

Original Research Article

In-vitro anthelmintic activity of *Boswellia serrata* and *Aloe barbadensis* extracts on *Pheretima posthuma*: Indian earthworm

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ABSTRACT

Background: The development of anthelmintic resistance and the high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants as an alternative source of anthelmintics. In the current study, in-vitro experiments were conducted to determine the possible anthelmintic effects of crude aqueous and alcoholic extracts of the resins of *Boswellia serrata* and leaves of *Aloe barbadensis* on adult Indian earthworm (*Pheretima posthuma*).

Methods: Various concentrations (50, 100, 150 mg/ml) of each extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. The activities are well compared with the standard drug Albendazole as a positive control and saline water as negative control.

Results: Anthelmintic activity was observed as dose dependent manner. It was found that alcoholic extract exhibited maximum anthelmintic activity at concentration 100 and 150 mg/ml compared to standard drug Albendazole (10mg/ml) while aqueous extract show modest significant activity at concentration 150 mg/ml against worm *Pheretima posthuma*. All results was statistically analysed by using 'Dunnett's test' one-way ANOVA; the $p < 0.001$ were significant when compared with control and standard group.

Conclusions: The present study proves the potential of combination of *B. serrata* and *A. barbadensis* as an anthelmintic drugs. Further studies are necessary to isolate and reveal the active compounds and to establish the mechanism of action.

Keywords: Anthelmintic, *Aloe barbadensis*, *Boswellia serrata*, Earthworm, *Pheretima posthuma*

INTRODUCTION

Helminths infections, generally known as helminthiasis is the infection of the human body with a parasitic worm such as roundworms and pinworms.¹ These parasitic worms can infect the tissues and intestines of a person. The worms usually only involve the intestinal tract but sometimes they may invade other organs. The type and severity of symptoms is determined by the type of worm and the part of the body infected.² Symptoms of helminth infections include nausea, vomiting, abdominal pains, confusion, and gastrointestinal bleeding. Helminthiasis causes a large threatening to the public health and lead to the prevalence of malnutrition, anemia, eosinophilia and pneumonia in developing countries.³ Anthelmintics are

drugs that expel parasitic worms (helminths) from the body, by either striking or killing the worms. Due to the high cost of currently available anthelmintic drugs and also the gastro-intestinal helminthes becomes resistant to the drugs; the treatment of helminthes diseases is one of the foremost problems.⁴

Therefore the research on herbal remedies as alternative anthelmintics has been increased. As medicinal plants play a rich source of anthelmintic agents, they are used medicinally in different countries for the treatment of helminths infections.⁵ India is one of the countries having rich biodiversity and huge number of herbal plants. *Boswellia serrata*, or *Shallaki*, is a tropical plant belonging to the family- *Burseraceae*. They are

moderate sized flowering plants, both trees and shrubs.⁶ The plant produces fragrant resin, and is used to make frankincense. It is widely used in traditional medicine, and forms an important part of ayurvedic medicine.⁷ In the historical documents the plant was used in the treatment for osteoarthritis, rheumatoid arthritis, joint pain, inflammation, Crohn's disease, abdominal pain, asthma, hay fever. It is also used as a stimulant, to increase urine flow, and for stimulating menstrual flow.⁸ *Boswellia serrata* shows its superiority over conventional drugs and is absolutely free from any toxic and side effects.

Aloe vera is a member of the lily family, and is a succulent plant that contains a lot of liquid in its thick, spiny leaves.⁹ It gives so many medicinal activities and pharmacological effects for human beings and animal. It acts as a natural fighter against all classes of infection, an important effective anti-oxidant, helps in treating all digestion related problems, heartburns, arthritis, stress, kidney-stone, skins-burns, diabetes, rheumatism, pain, asthma, cancer, AIDS, It also acts as a laxative beauty enhancer and produced that effect on lowering blood sugar level in diabetics and maintain the blood sugar.¹⁰ It is commonly known as Barbados or Curaçao Aloe, is an herbal medicine with a long traditional use in different cultures.¹¹ Literature survey reveals that till date no reports were found on the anthelmintic activity of above plant extracts in combination. Therefore it was thought worthwhile to explore these two indigenous plants for their activity against the Indian earthworm.

METHODS

Plant material

Herbal extract powders of *Boswellia serrata* and *Aloe barbadensis* are obtained as a gift sample from Sane Guruji Hospital and Ayurvedic Medical Store, Hadapsar (Pune) in the month of February 2020.

Chemicals

Albendazole (Bendex) obtained from cipla company. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

Preparation of aqueous extract

The dried powder (10g) was extracted with water for 24 hr and the same was dried on water bath.¹²

Preparation of ethanol extract

The dried powder (10g) extracted in a soxhlet apparatus using ethanol (95%), at a temperature range of 45°C to 60°C. The filtrate was evaporated to dryness at reduced pressure in vacuum evaporator and stored in freeze for further study.¹²

Experimental Animal

Adult earthworms (*Pheretima posthuma*) was used to evaluate anthelmintic activity in vitro. Earthworms were collected from the damp soil and washed with normal saline to remove all the faecal matter. *P. posthuma* with 6-8 cm in length and 0.3-0.5 cm in width was used for anthelmintic activity test. The worms were identified in the Department of Zoology, Waghire College, Saswad. This organism was selected as a model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.¹³ Before initiation of experiment the earthworms were washed in normal saline.

Inclusion criteria

- Healthy earthworms having length 6-8cm were included in the study.
- Earthworms with same species included in this study to avoid error in results.

Exclusion criteria

- Length >8cm, those terminally ill, non-healthy, unconscious, injured and different species of Earthworm should be excluded from the study.

Anthelmintic activity on *Pheretima posthuma*

This was an *in-vitro* study carried out in the department of pharmacology of pharmacy college, Saswad in the month February 2020. The anthelmintic activity was performed according to the method followed by Ajaiyeoba et al, with minor modifications.^{14,15} On adult Indian earth worm *Pheretima posthuma*, as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Albendazole was used as standard reference (positive control) and saline water as negative control. *Pheretima posthuma* was placed in petridish containing three different concentrations (50,100 and 150 mg/ml) of alcoholic and aqueous extract of test drug. Each petridish was placed with 2 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with standard drug Albendazole (10 mg/ml) treated samples. All the results were shown in Table 1 and 2 and expressed as a mean±SEM of two worms in each group.

Statistical analysis

The results are presented as mean±SEM (Standard Error of Mean). Statistical analyses graphical representations of results for anthelmintic study were evaluated by one-way ANOVA following Dunnett's Multiple Comparisons test

using Graph Pad Prism version 8.4 for windows. Graph Pad Software, San Diego California USA, the $p < 0.001$ was considered to be statistically significant.

RESULTS

Preliminary Phytochemical screening

The phytochemical screening revealed that alcoholic and aqueous extract contain gums and mucilage, resins, flavonoids, alkaloids, steroids, tannins, glycosides and saponins.

Anthelmintic activity

The *B. serrata* and *A. barbadensis* extracts produced a significant anthelmintic activity in dose dependent manner as shown in Table 1 and 2. Alcoholic extract took less time to cause paralysis and death of the earthworms as compared to aqueous extract. Thus alcoholic extract was found to be more potent than aqueous extract (Figure 3 and 4). Data (Table 1 and 2) reveals that all the three concentrations of aqueous and alcoholic extract (50, 100 and 150 mg/ml) show better and potent anthelmintic activity than that of standard drug Albendazole.

Table 1: Effect of aqueous BS+AB extracts in earthworms.

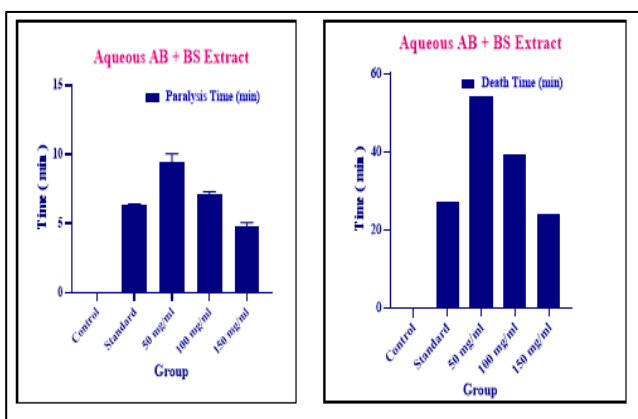
Group	Treatment	Concentration (mg/ml)	Time Required to Paralyze (min)	Time Required to Death (min)
I	Negative Control (Normal saline)	-	-	-
II	Positive Control (Albendazole)	10	6.3±0.14***	27.0±0.70****
III	Aqueous BS + AB Extracts	50	9.4±0.63***	54.1±0.14****
		100	7.15±0.17***	39.5±1.06****
		150	4.8±0.28**	24.15±0.67****

All the values are expressed as mean ± SEM, N=2).

Table 2: Effect of Alcoholic BS + AB extracts in earthworms.

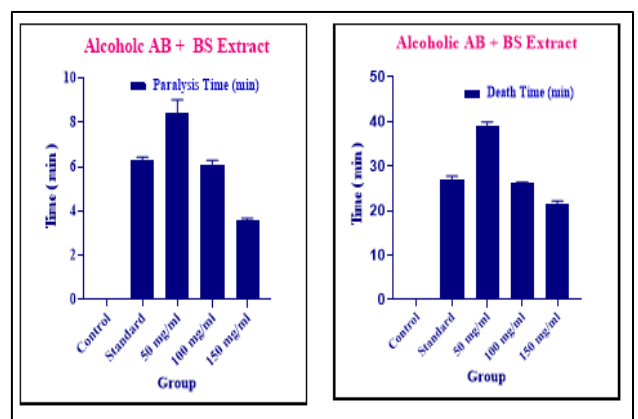
Group	Treatment	Concentration (mg/ml)	Time Required to Paralyze (min)	Time Required to Death (min)
I	Negative Control (Normal saline)	-	-	-
II	Positive Control (Albendazole)	10	6.3±0.14***	27.0±0.70***
III	Alcoholic BS + AB Extracts	50	8.4±0.63***	38.9±0.91 ***
		100	6.1±0.19 ***	26.1±0.28 ***
		150	3.6±0.07 **	21.3±0.84****

(All the values are expressed as mean ± SEM, N=2).



Each value represents the mean±SEM , n=2, ****p<0.0001, compared with control, Dunnett’s Multiple Comparisons test after analysis of variance.

Figure 1: Graphical representation for the anthelmintic activity of AB + BS aqueous extract compared to standard drug Albendazole (Paralysis and Death).



Each value represents the mean ± S.E.M., n = 2, ***P < 0.001, compared with control, Dunnett’s Multiple Comparison test after analysis of variance.

Figure 2: Graphical representation for the anthelmintic activity of AB + BS alcoholic extract compared to standard drug Albendazole (Paralysis and Death).

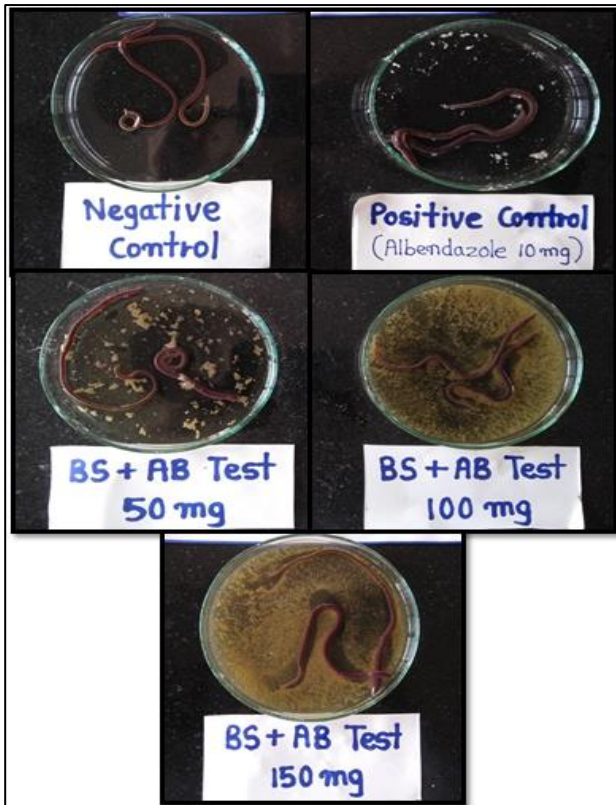


Figure 3: Anthelmintic Activity of BS + AB Aqueous Extract.

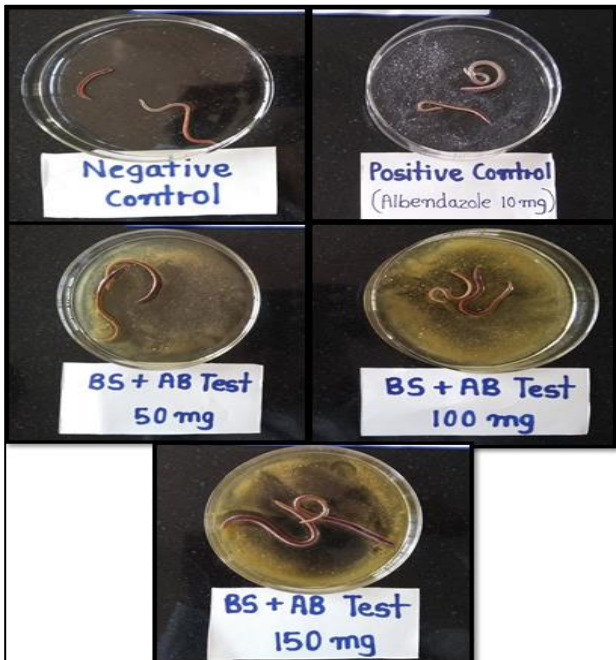


Figure 4: Anthelmintic Activity of BS + AB Alcoholic Extract.

The above findings justify the anthelmintic properties of BS+AB extract. Alcoholic extract of BS + AB at 100 mg/ml concentration shows paralysis at 6.1 min and death 26.1 min, and also at 150 mg/ml concentration

shows paralysis at 3.6 min and death 21.3 min respectively whereas aqueous extract of BS + AB at 150 mg/ml show paralysis at 4.8 min and death 24.15 min by the earth worm *Pheretima posthuma*. The reference drug Albendazole exhibited the same and show paralysis at 6.3 min and death 27 min respectively (Table 1 and 2). Albendazole exhibits anthelmintic activity by blocking glucose uptake and depletion of glycogen stores in test parasite. The alcoholic and Aqueous extracts of *B. serrata* and *A. barbadensis* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 150 mg/ml in shorter time as compared to that of Albendazole (Figure 1 and 2).

DISCUSSION

Preliminary phytochemical screening of extract revealed the presence of glycosides, flavonoids, phenolic compounds and mucilage. The anthelmintic effect was depend to the phytochemical constituent present in each extract. These results may be attributed to the content of polyphenol compounds which were higher in ethanolic extract compared to aqueous extracts. Ethanol solvent had better characteristics in penetrating cell walls, causing the release of high concentrations of polyphenol from plant materials.¹⁶ The low anthelmintic activity of the aqueous extract against *P. posthuma* worm may be resulted from the activity of the polyphenol oxidase enzyme which is able to reduce polyphenols levels in the aqueous extract, whereas in ethanol solvent, this enzyme is inactive. The extraction process also used heat that degraded the active compounds. Several studies had also reported that higher flavonoid compounds were detected when extracted using 95% ethanol which has higher polarity than pure ethanol.¹⁷

CONCLUSION

The alcoholic extract gave better results which may be due to the higher concentration of gum and mucilage and resins as compared to aqueous extract. It can be concluded that both alcoholic and aqueous extract of BS + AB combination possess synergistic anthelmintic effect. Thus, both herbal plants can be used in controlling the diseases caused by worms. In conclusion, the present study proves the potential of combination of *B. serrata* and *A. barbadensis* as an anthelmintic drugs. Further studies are necessary to isolate and reveal the active compounds and to establish the mechanism of action.

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Ethical approval: Not required

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