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***Research article***

# ANTIHELMINTIC ACTIVITY OF STEM BARK EXTRACT OF *BRIDELIA RETUSA* SPRENG.

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**ABSTRACT:** The present study was undertaken to investigate antichelmintic activity of petroleum ether, chloroform, ethanol and aqueous extract of the stem bark of *Bridelia retusa* S. on adult African night crawlers (*Eudrilus euginae*) earthworms due to its anatomical and physiological similarity with the intestinal roundworm parasites of human being. Antihelmintic activity was investigated at 50 mg/ml for all the four extracts on earthworms and compared with standard piperazine citrate. The time of paralysis and death of the earthworms for piperazine citrate, petroleum ether, chloroform, ethanol and aqueous extract was noted. The death of earthworms occurred within few minutes of their paralysis. However, in control group, worms were observed for 24 hours and no paralysis or death was found during that period. In the present study, the extracts obtained using polar and non polar solvents were used for antihelmintic activity against earthworms. Comparing all extracts and standard of piperazine citrate at the same concentration (50 mg/ml), it was clear that chloroform extract showed significantly ($p<0.05$) better effect and hence higher antihelmintic activity in comparison to petroleum ether, ethanol and aqueous extract as well as that of standard, followed by ethanol extract which showed similar effects to standard while the rest of the extract showed poor effects. Thus indicating antihelmintic properties of the extract which may be attributed to the phytoconstituents present in it which needs further investigation.

**Keywords:** *Bridelia retusa* Spreng, sequential extraction, antihelmentic activity.

## INTRODUCTION

Helmintic infections are among the most common infections in man, affecting a large proportion of population all over the world. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas, and some of them can be developed in temperate climates (Bundy, 1994). Parasitoses have been of concern to the medical field for centuries and the helminthes still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anithelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health (Patel *et al.*, 2011). Parasitic diseases cause severe morbidity affecting mainly population in endemic areas with major economic and social consequences. The gastro-intestinal helminthes becomes resistant to currently available antihelmintic drugs therefore, there is a foremost problem in treatment of helminthes diseases; hence there is an increasing demand towards natural antihelmintics. Tremendous progress has been made in the development of antihelmintic drugs in the past 50 years. During this period, the current classes of synthetic drugs were developed, including the benzimidazoles and imidazothiazoles (such as levamisole). Another major step was achieved with the introduction of the avermectin class of macrolactones, the discovery of this compound class led to antihelmintic drugs, such as ivermectin and doramectin, which have excellent broad-spectrum activity and superior potency. However, resistance to all of these classes of drugs has been observed, leading to the continuing need for further research to discover new classes of antihelmintics, especially those with novel modes of action.

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The development of naturally-occurring compounds as medicines for human use and for treatment of animals is fraught with problems (Behnke *et al.*, 2008). It would, therefore, be imperative to explore possibilities of developing new antihelmintic compounds. This has drawn attention of researchers to the validation of traditionally used botanical antihelmintics (Hammond *et al.*, 1997; Akhtar *et al.*, 2000; Waller *et al.*, 2001; Iqbal *et al.*, 2003). Because of easy availability, earthworms have been used widely for the evaluation of antihelmintic compounds *in vitro* (Jain and Jain, 1972; Sallaman, 1981; Dash *et al.*, 2002; Shivkar and Kumar, 2003). Few species of plants recognized as antihelmentics include *Embelia ribes*, *Chenopodium ambrosioides*, *Dryopteris* sps and *Artemisia* sps (Prakash and Mehrotra, 1987). A study revealed a trypanocidal potential in methanolic extract of *B. ferruginea* stem bark which was attributed to the presence of alkaloids, tannins saponins, steroids and phlobatanins (Ekanem *et al*., 2008) Hence, the present study on the bark material was undertaken to study the antihelmentic activity of stem bark extracts of *B. retusa* S.

## MATERIALS AND METHODS

### Plant material

The bark of *Bridelia retusa* S. was obtained from the jungles of Western Ghats (Amboli) of India under the guidance of Forest Officer (Forest Department, Collem, Goa). The plant material was cut into pieces and subjected to shade drying. On complete drying the pieces were powdered mechanically (by means of mortar and pestle) and stored in air tight containers at room temperature for future use.

### Antihelmintic assay

The antihelmintic activity of the petroleum ether, chloroform, ethanol and aqueous extracts of bark of *B. retusa* was determined by using the method of Patra *et al*. (2008). The activity was evaluated on adult African night crawlers (*Eudrilus euginae*) earthworms due to its anatomical and physiological similarity with the intestinal roundworm parasites of human being. The earthworms were obtained from University of Agricultural Sciences (UAS, Dharwad) along with compost. They were washed in saline, earthworms of uniform length were chosen for the study. The extract solution containing each of petroleum ether, chloroform, alcoholic and aqueous extract (50 mg/ml in normal saline) were prepared and ten worms were placed in it. Piperazine citrate (50 mg/ml) was used as reference standard while normal saline as control. The time taken for paralyses and death of individual worms was observed. Paralysis was noted when the worms became immobile even in the normal saline solution. Death was concluded when the worms lost their motility followed by fading away of their body color.

## RESULTS AND DISCUSSION

Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths (Geert and Dorny, 1995; Coles, 1997) against antihelmintics have led to the proposal of screening medicinal plants for their antihelmintic activity. In the present study, the extracts obtained using polar and non polar solvents were used for antihelmintic activity against earthworms. Using all extracts and standard of piperazine citrate of same concentration (50 mg/ml), it was clear that chloroform extract showed significantly better effect and hence higher antihelmintic activity in comparison to petroleum ether, chloroform and aqueous extract as well as that of standard. The petroleum ether and chloroform extract of leaves of *Benincasa hispida* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml, in shorter time as compared to reference drug piperazine citrate, alcoholic and aqueous extracts (Bhattacharjee *et al*., 2010) as also seen in the present study with best results for chloroform extract. The ethanolic extract of the drupes of *Melia azedarach* L. was active against both the tapeworm and the earthworm tested and found to be comparatively more active than piperazine phosphate against *Taenia solium* (Szewczuk *et al*., 2003) as also seen in chloroform extract of the present study.

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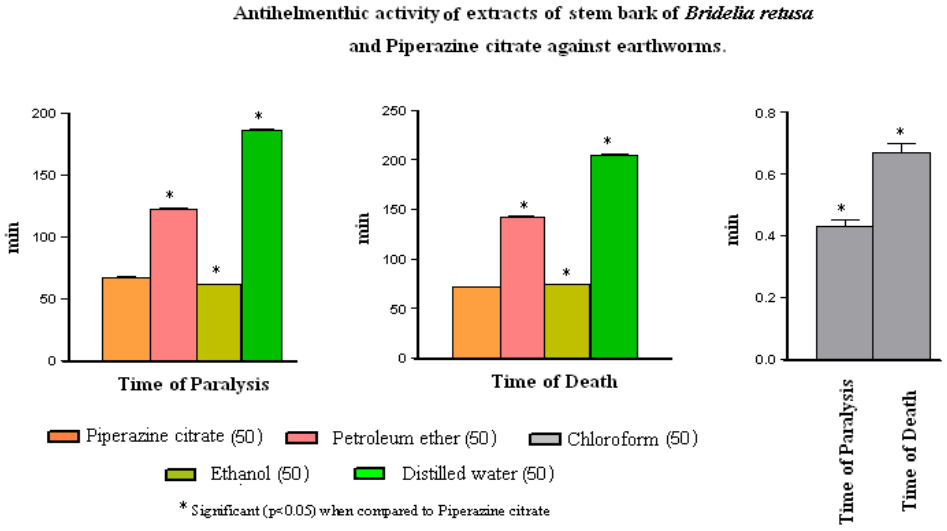
The antihelmintic activity of *Rhynchosia minima* may be attributed to the presence of active components such as flavonoids, tannins and terpenoids, the antihelmintic properties of which are well documented (Niezen et al., 1995). The methanolic extract of *Indigofera tinctoria* displayed a significant antihelmintic property in a dose dependent manner giving shortest time of paralysis and death with 100 mg/ml concentrations, comparable with the standard drug (Balamurugan and Selvarajan, 2009) as seen at 50 mg for chloroform extract in the present study. From the results it is observed that *Moringa oleifera* showed potent antihelmintic activity along with *Vitex negundo* (Rastogi *et al*., 2000). It was concluded that active constituents responsible for antihelmintic activity was present in the ethyl acetate and petroleum ether extracts of seeds of *Pongamia glabra* comparable to standard drugs (Nirmal *et al*., 2007) which may be present in the chloroform extract of the present study. The antihelmintic activity of *Rhynchosia minima* may be attributed to the presence of active components such as flavonoids, tannins and terpenoids, the antihelmintic properties of which are well documented (Niezen *et al.,* 1995). This indicates that the antihelmintic principles are non polar compounds as also seen in the present study. The results of antihelmintic activity revealed that petroleum ether, chloroform, ethanol and aqueous extracts exhibited varying degree of activity against the earthworms and caused paralysis followed by death which may be attributed to the presence of different phytoconstituents. The possible mechanism of the antihelmintic activity of *Asta churna* from previous results may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis (Devi *et al*., 2009) which may be so in the present study as well. Results reported in the resent work constitute rational evidence and a scientific basis to justify and support the folklore claims of the potential antihelmintic activity of *B. retusa*. Further studies are required on phytochemical profiling as well as isolation and identification of bioactive component responsible for antihelmintic activity.(Table No-1, Fig-1)

**Antihelmentic activity of extracts of stem bark of *Bridelia retusa* and piperizine citrate against earthworms.**

| Sr. No | Concentration of extracts and Piperazine citrate. (50 mg/ml, respectively) | Time of Paralysis (min) | Time of Death (min) |
|---|---|---|---|
| 1 | Control | --- | --- |
| 2 | Piperazine citrate | 69.60±0.87 | 71.14±0.27 |
| 3 | PE | 122.20±0.97* | 142.05±0.95* |
| 4 | C | 0.43±0.02* | 0.67±0.03* |
| 5 | E | 61.25±0.35* | 74.19±0.43* |
| 6 | D W | 185.42±1.35* | 204.65±0.53* |

Control: Normal Saline, PE: Petroleum ether, C: Chloroform, E: Ethanol, DW: Distilled Water. (-): No activity. * Significant vs Piperazine citrate ($p<0.05$)



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## CONCLUSION
The antihelmintic activity was maximum in chloroform extract which was better than standard and in comparison to other extract followed by ethanol extract which showed similar effects to standard while the rest of the extract showed poor effects, all the extracts were compared with standard drug at 50 mg/ml. Earthworms were chosen in the present study due to their resemblance to human and animal gut parasites. The death of earthworms occurred within few minutes of their paralysis. This study was performed to evaluate antihelmintic activity of the plant which may help to discover new chemical classes of drugs that could serve as selective chemotherapeutic agents for the maintenance of health. The promising results obtained indicate extensive studies on the plant will enable to explore its potentials. Thus, this study justifies the folklore medicinal and therapeutic value of the plant.

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