Study on of ethanol and aqueous extracts of *Embelia ribes* for anthelmintic properties

ABSTRACT

Objectives: The present research study was conducted for the comparative evaluation of ethanol and aqueous extract of *Embelia ribes* for its *in vitro* anthelmintic properties. **Methods**: The authenticated leaves of plant *Embelia ribes* were dried and powdered. The powdered drug was defatted with petroleum ether and a part of marc leftover was subjected to ethanol extraction using Soxhlet apparatus. Another part was subjected aqueous extraction using chloroform water. The ethanol and aqueous extracts of *Embelia ribes* was subjected to preliminary phytochemical investigation. Both the extracts were evaluated for in vitro anthelmintic properties using earthworms. **Results**: The ethanol and aqueous extracts were revealed for the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins and phenols. The paralysis time and death time of worms were significantly reduced by the administration of ethanol and aqueous extracts of *Embelia ribes*. **Conclusion**: The result of the present investigation confirms the anthelmintic property of *Embelia ribes* .

Key words: Anthelmintic activity, *Embelia ribes*, piperazine citrate, *Pheretima posthuma*, earthworms.

1.0 INTRODUCTION

"Pathogens with significant worldwide significance include parasitic worms (helminths) of the gastrointestinal (GI) tract. According to estimates, more than a billion people, mostly in developing nations, are infected with helminths that are spread through the soil. Helminth infection also poses a substantial danger to global food security and is a major concern for livestock production" [1,2]. "A few handful of synthetic anthelmintic medications are essentially the only means of controlling helminths. The risks of parasites developing drug resistance (which

is already common in some livestock production systems), the cost of drugs for small-scale farmers in developing countries, and for some helminths, the lack of efficacy of currently available drugs are the drawbacks of this reliance on chemotherapy. Therefore, there is an urgent need for additional and supplementary helminth control methods" [3,4]. Humans and animals have historically used natural plant extracts as dewormers, but there hasn't been much scientific research to support this use or identify the active ingredients [5,6]. Plants' anthelmintic properties are typically attributed to secondary metabolites such proanthocyanidins, sometimes known as condensed tannins, alkaloids, terpenoids, or polyphenols [7,8]. Embellin, Procyanidins, prodelphinidins, and other proanthocyanidins are a diverse and common class of chemicals that are made up of polymers of catechin, epicatechin, and/or gallocatechin, with hetero-polymers being frequent. They have been extensively researched for their antioxidant and anti-inflammatory activities and can be found in both tropical and temperate plant material [9,10]. "Since ancient period Ayurveda physicians Charka and Sushruta had mentioned the usefulness of several medicinal plants for the effective management diabetes with fewer side effects in Ayurveda, the traditional medicinal system of India. Herbal remedies for diabetes mellitus constituting of plant substances, either a single agent or in combination with other drugs, which are considerably safe and free from adverse reactions compared to synthetic agents" [10]. "*Embelia ribes* is an important medicinal plant that is used in a long period of time as herbal remedy to treat various diseases and ailments" [11]. "Different parts of the plant such as the stem, leaves, roots, seeds and barks are widely accepted worldwide in traditional setting and used as various form of remedies such as diuretic, astringent, anti-inflammatory, antibacterial, antihelmintic, etc. Modern researchers however showed that the various plant extracts has exhibited many potential as antibacterial, anti-fungal, anti-inflammatory, analgesic, antitumor

and contraceptive ability" [12]'. These attributes are both related to an array of phytochemicals that are embedded within the plant parts for anthelmintic activities[13]. Hence, the present study is designed to test effect of ethanol extract of *Embelia ribes* for anthelmintic properties against earthworms.

2.0 MATERIALS AND METHODS

2.1 Plant material

The areal part of *Embelia ribs* was collected from Foundation for Revitalization of Local Health Traditions (FRLTH) No.74/2, Jarakabande Kaval, Post Attur, Via Yelahanka, and Bangalore, Pin Code: 560106 Karnataka, INDIA. The plant was authenticated by Dr. Rama Rao, Scientist, Regional Ayurveda Research Institute for Metabolic Disorders.

2.2 Preparation of the ethanol extract

The leaves of the plant are dried under shade. The dried leaves are then powdered and 200 g of powdered drug was defatted with petroleum ether. A part defatted powdered drug was subjected to ethanol extraction in soxhlet apparatus for 48 hours and second part was subjected to aqueous extraction using chloroform water[13].

2.3 Preliminary phytochemical investigation

The preliminary phytochemical investigation for the ethanol (EEER) and aqueous extract (AEER) of *Embelia ribes* was conducted as per procedure prescribed by Khandelwal[14].

2.4 Evaluation of in vitro anthelmintic activity of extract of *Embelia ribes*

Collection of Indian earthworms: "Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil, the average size of earthworm being 6-8 cm. They were washed with tap water for the removal of the adhering dirt. The average sizes of the worms were 5-6 cm. The anthelmintic assay was performed on adult Indian earthworm *Pheretima*

posthuma, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug" [15,16]: The study consisting of 6 groups as follows

- **Group I-Normal**: Consisting of 6 worms treated with normal saline
- **Group II-Standard**: Consisting of 6 worms treated with 3 % of piperazine citrate
- **Group III-EEER-low dose**: Consisting of 6 worms treated with 1 % of ethanol extract of *Embelia ribes.*
- **Group IV-EEER-medium dose**: Consisting of 6 worms treated with 3 % of ethanol extract of *Embelia ribes.*
- **Group V-EEER-high dose**: Consisting of 6 worms treated with 5 % of ethanol extract of *Embelia ribes.*
- **Group VI-AEER-low dose**: Consisting of 6 worms treated with 1 % of aqueous extract of *Embelia ribes.*
- **Group VII-AEER-medium dose**: Consisting of 6 worms treated with 3 % of aqueous extract of *Embelia ribes.*
- **Group VIII-AEER-high dose**: Consisting of 6 worms treated with 5 % of aqueous extract of *Embelia ribes.*

The assay was performed on adult Indian earth- worm due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings.Various dilutions ofstandard drug (Piperazine citrate) and testwere prepared in normal saline (0.85%). Different concentrations of standard drug and test compounds in normal saline were poured into respective labeled Petri plates (50 ml in each plate) and 6 worms of equal size (or nearly equal) were introduced into each of the plates.

Parameters: Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was con- cluded when the worms lost their motility followed with fading away of their body colors. Death was also confirmed bydipping the wormsin slightlywarmwater. The mortality of parasite was assumed to have occurred when allsigns of movement had ceased.

Statistical Analysis

The data obtained from the present investigation were analyzed by ANOVA followed by post hoc Dunnet's t-test with the help ofGraphpad prism5 software. All the values were shown as mean±standard error of mean (S.E.M.).

3.0 RESULTS

2.1 Preparation of extracts

The percentage yield of ethanol and aqueous extracts of *Embelia ribes* were found to be 9.09 and 8.22 respectively.

2.2 Preliminary phytochemical study

The study revealed that, the ethanol and aqueous extracts of *Embelia ribes* consists of glycosides, alkaloids, flavonoids, tannins and phenolic compounds.

Table 1: Results of primary phytochemical analysis on EEER

Sl. No	Phytoconstituent present	Presence
1.	Carbohydrates	++
2.	Alkaloids	++
3.	Flavonoids	++
4.	Glycosides	++
5.	Tannins	+
6.	Polyphenols	+++
7.	Proteins	++

2.3 Evaluation of in vitro anthelmintic activity of EEER and AEER

The results of anthelmintic activity of different concentrations of ethanol and aqueous extract of *Embelia ribes* are depicted in Table no. 2. The results revealed concentration dependent

anthelmintic activity for both the extracts. The average paralysis time (in min) in different concentrations of Standard drug (Piperazine citrate) was found to be 21 mins while the average death time (in min) was found to be 31 mins. Ethanol extract of *Embelia ribes* in 1%, 3% and 5% was found to cause paralysis of worms in 73, 40 and 23 minutes respectively followed by death in 85, 47 and 28 minutes respectively. For aqueous extract of *Embelia ribes* in 1%, 3% and 5% was found to cause paralysis of worms in 68, 28 and 18 minutes respectively followed by death in 74,30 and 25 mins respectively and observed death in 74, 30 and 25 mins respectively . There were significant reduction in paralysis and death time were found in extracts treated group when compare to normal. The effect was comparable to that of piperazine citrate [Table 2 Fig 1-2].

Table 2: Effect on EEER & AEER on of paralysis time and death time of earthworms

Sl.No.	Group	Paralysis Time (mins)	Death Time (mins)
I.	Normal	130.55±3.08	159.79±8.84
II.	Standard	21.57***±1.48	31.56***±3.29
III.	EEAR-Low dose	73.34**±6.82	85.22**±6.00
IV.	EEAR-Medium dose	40.52***±3.27	47.25***±2.68
V.	EEAR-High dose	23.08***±2.16	28.81***±2.52
VI.	AEAR-Low dose	68.51**±4.22	74.37**±5.54
VII.	AEAR-Medium dose	28.23***±1.62	30.83***±5.74
VIII.	AEAR-High dose	18.88***±1.01	25.59***±3.55

Figure 1: Effect on EEER & AEER on time paralysis time of earthworms

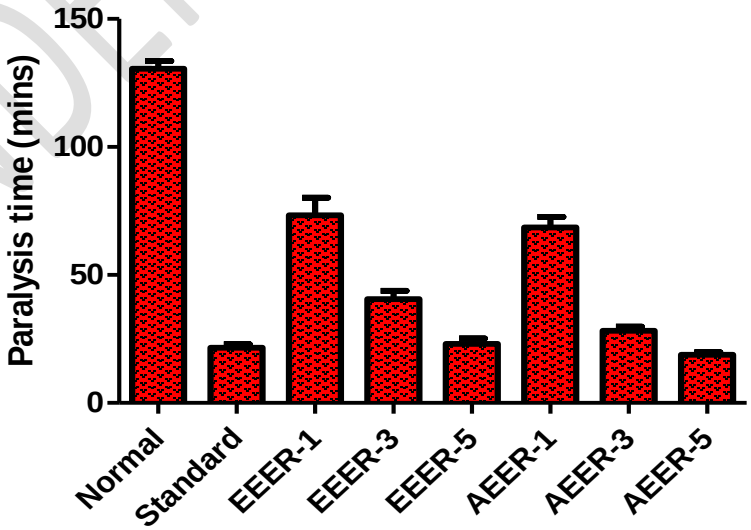
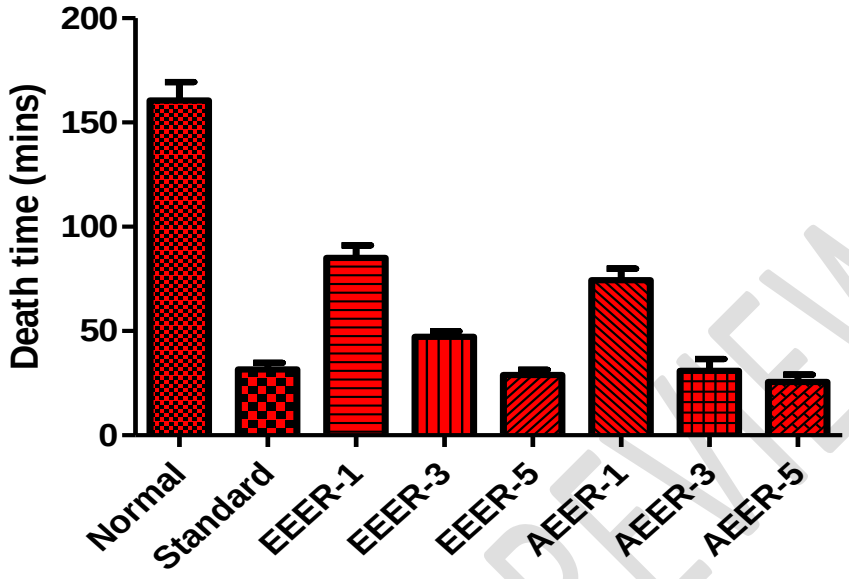


Figure 2: Effect on EEER & AEER on death time of earthworms



4.0 DISCUSSION

Although there are a number of pharmacological medicines available for the treatment of helminthiasis, none of them are completely adequate for the disease's successful and side-effect-free management. Therefore, it is still quite desirable to find and create novel therapeutic drugs. Since herbal medicine plays a crucial role in this segment due to their minimal side effects, searching for more potent and less toxic anthelmintic drugs from plant origin is under pipeline throughout the world. This is due to the toxicities effects and adverse reactions associated with the therapy using the currently available anthelmintic drugs.

The present research study was conducted to evaluate ethanol and aqueous extract of *Embelia ribes* for its *in vitro* anthelmintic properties. The authenticated leaves of plant *Embelia ribes* were dried and powdered. The powdered drug was defatted with petroleum ether and a part of marc leftover was subjected to ethanol extraction using Soxhlet apparatus. Another part was subjected aqueous extraction using chloroform water. The ethanol and aqueous extracts of *Embelia ribes* was subjected to preliminary phytochemical investigation. Both the extracts were evaluated for in vitro anthelmintic properties using earthworms. The ethanol and aqueous extracts were revealed for the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins and phenols. The paralysis time and death time of worms were significantly reduced by the administration of ethanol and aqueous extracts of *Embelia ribes*.

5.0 CONCLUSION

The result of the present investigation confirms the anthelmintic property of ethanol and aqueous extracts of *Embelia ribes*. But the aqueous extract was more effective than ethanol extract. Further study should be conducted isolate and test specific constituents responsible for the anthelmintic activities and also to determine their mechanism of action.

6.0 REFERENCES

1. Charlier J, van der Voort M, Kenyon F, Skuce P, Vercruysse J: Chasing helminths and their economic impact on farmed ruminants. Trends Parasitol 2014, 30(7):361-67.
2. Fitzpatrick JL: Global food security: The impact of veterinary parasites and parasitologists. Vet Parasitol 2013, 195(3–4):233-48.
3. Sutherland IA, Leathwick DM: Anthelmintic resistance in nematode parasites of cattle: a global issue? Trends Parasitol 2011, 27(4):176-81.
4. Sargison ND: Pharmaceutical treatments of gastrointestinal nematode infections of sheep-Future of anthelmintic drugs. Vet Parasitol 2012;189(1):79-84.
5. Githiori JB, Höglund J, Waller PJ: Ethnoveterinary plant preparations as livestock dewormers: practices, popular beliefs, pitfalls and prospects for the future. Anim Health Res Rev 2005; 6(01):91-103.
6. Athanasiadou S, Githiori J, Kyriazakis I: Medicinal plants for helminth parasite control: facts and fiction. Animal 2007;1(09):1392-400.
7. Tolossa K, Debela E, Athanasiadou S, Tolera A, Ganga G, Houdijk J: Ethnomedicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. J Ethnobiol Ethnomed 2013;9(1):32-4.
8. Cowan MM: Plant Products as Antimicrobial Agents. Clin Microbiol Rev 1999;12(4):564–82.

9. Mueller-Harvey I: Unravelling the conundrum of tannins in animal nutrition and health. J Sci Food Agric 2006, 86(13):2010-37.
10. Martinez-Micaelo N, González-Abuín N, Ardèvol A, Pinent M, Blay MT: Procyanidins and inflammation: Molecular targets and health implications. Biofactors 2012, 38(4):257–65.
11. González R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A, Martínez- Augustin O, et al. Effects of flavonoids and other polyphenols on inflammation. Crit Rev Food Sci Nutr 2011;51(4):331-62.
12. Amin H. Antimicrobial activity of Vidanga Churna (*Embelia ribes* Burm .) by cup diffusion technique. Pharmagene 2017;3(2):1–3.
13. Kokate CK. Practical Pharmacognosy. VallabhPrakashan New Delhi: 1994;4:110-1.
14. Khandelwal KR, Practical Pharmacognosy-techniques and experiments. Pune; NiraliPrakashan; 2000.
15. Aziz A, Sarwar Raju G, Das A, Ahmed J, Moghal MM. Evaluation of In vitro Anthelmintic Activity, Total Phenolic Content and Cytotoxic Activity of *Crinum latifolium* L. (Family: Amaryllidaceae). Adv Pharm Bull. 2014;4(1):15-9.
16. Das SS, Dey M, Ghosh AK. Determination of anthelmintic activity of the leaf and bark extract of *Tamarindus indica* linn. Indian J Pharm Sci. 2011 Jan;73(1):104-7.