

# Evaluation of *in-vitro* Anthelmintic Activity of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylon*

Arun Kashivishwanath Shettar and Ankala Basappa Vedamurthy\*

## ABSTRACT

**Objective:** Evaluating Anthelmintic activity of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylon* extracts by using *in vitro* assay. **Methods:** The serial exhaustive extraction was carried out with a series of solvents: chloroform, ethyl acetate, methanol, ethanol and water with increasing polarity using Soxhlet apparatus. The concentrated and dried extracts were evaluated for anthelmintic activity by employing standard *in vitro* method (*Pheretima Posthuma* model). **Results:** *In vitro* anthelmintic study shows that in case of *Ximenia americana* chloroform extract showed higher anthelmintic activity where as in case of *Hopea ponga* and *Vitex leucoxylon* methanol extract exhibited significant activity when compared to other solvent extracts. **Conclusion:** Results confirm that methanol extract of *Hopea ponga* exhibited highest anthelmintic activity among all tested extracts. This study provides scientific evidence that the leaves of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylon* have anthelmintic efficacy. However further comprehensive chemical and pharmacological investigation should be carried out to isolate the active compounds and appropriate elucidation of its mechanism of action and it helps in the development of new pharmaceuticals to treat Helminthiasis.

**Key words:** *Ximenia americana*, *Hopea ponga*, *Vitex leucoxylon*, *Pheretima Posthuma* model, *in vitro* anthelmintic activity.

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### History

- Submission Date: 26-12-16;
- Review completed: 05-01-17;
- Accepted Date: 02-02-17.

DOI : 10.5530/pj.2017.3.62

### Article Available online

<http://www.phcogj.com/v9/i3>

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## INTRODUCTION

Helminthiasis is an infection disease caused by nematode worms such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Nectator americanus* and *Ancylostoma duodenale*. Infection occurred when ingesting food contaminated eggs or larvae, hands or utensils or through penetration of the skin by infective hook-worm larvae in contaminated soil.<sup>1</sup> According World Health Organization (WHO) estimation more than 1.5 billion (24%) of world population are infected by parasitic worms. The parasite worm infection cases are commonly occurred in tropic and sub tropic regions with most cases in Africa, America, China and Southeast Asia.<sup>2</sup> Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for wide spread nature of this disease in the developing countries. In developing countries these parasite infections became threat to society by causing severe morbidity, including lymphatic filariasis, onchocerciasis and schistomomiasis.<sup>3</sup> As per World Health Organization (WHO), only few drugs are frequently used in the treatment of parasitic infections.<sup>4</sup> Anthelmintic drugs can be classified according to their chemical structure as well as their specific action towards specific type of the helminthes.<sup>5</sup> Because of the increasing toxicity and allergic manifestations of anthelmintic drugs the gastrointestinal helminthes became resistant to current available synthetic anthelmintic drugs.<sup>6,7</sup> From ancient time medicinal plants play important role in the elimination of soil

transmitted Helminthiasis. Natural products based drugs have used against various diseases since time immemorial. Plant derived natural products hold great promise for the discovery and development of new drugs. Medicinal plants provided with secondary metabolites usually called as phytochemicals or bioactive compounds which are considered as natural source of antioxidant, antimicrobial and anti-inflammatory agents which have been shown to reduce the risk and progression of many diseases such as cancer and diabetes<sup>8,9</sup> and also many plants have been used to treat parasitic infections in humans and animals.<sup>10-13</sup> In the present study, *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylon* plants were selected for *in vitro* anthelmintic study. *Ximenia americana* Linn. Plant belonging to Olacaceae family was selected. *X. americana* is a small tree or shrub, native to tropical area of Africa and seen distributed in many parts of the world. This species is used in treatment of wide variety of ailments by many rural communities in Africa and Asia. This is commonly known as wild olive or sour plum or yellow plum and extensively used as herbal remedy in treatment of malaria, leproutic ulcer, and skin infection.<sup>14</sup> The leaves are reported to have antibacterial activity and also used in the treatment of fever, tuberculosis, tooth decay and wounds.<sup>15</sup> *Hopea ponga* is an endemic tree belonging to Dipterocarpaceae family found in tropical ever green forest of western India and it is widely distributed along the Western Ghat of Karnataka.<sup>16</sup> *H.ponga* is categorized as an endangered tree spe-

**Cite this Article:** Shettar AK, Vedamurthy AB. Evaluation of *in-vitro* Anthelmintic Activity of *Ximenia Americana*, *Hopea ponga* and *Vitex leucoxylon*. Pharmacogn J. 2017;9(3):367-71.

cies under the International Union for Conservation of Nature Red List of threatened species. This plant was reported to be used as traditional medicine in the treatment of piles and snake bite.<sup>17</sup> Bark of *Hopea ponga* is known to have high content of tannin and acts as astringent.<sup>18</sup> *Vitex leucoxylo*n is commonly known as five leaved chaste tree and belongs to the verbenaceae family. It is small to large deciduous tree, growing up to 20 m in height. It is widely distributed along the Western Ghats of India. The leaves of *V.leucoxylo*n are reported to have medicinal properties like relieving headache, fever and catarrh.<sup>19</sup> Reports indicate that aqueous and ethanolic extracts of *V.leucoxylo*n leaves possess antipsychotic, antidepressant, analgesic, anti-inflammatory, anti-parkinsonian and antimicrobial activities.<sup>20, 21</sup> However these three above mentioned plants have not been subjected for investigation for their anthelmintic activity. With this background, the present study was undertaken to evaluate anthelmintic properties of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylo*n.

## MATERIALS AND METHODS

### Plant collection

Leaves of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylo*n were collected from Anashi forest range of Western Ghats, Uttara Kannada District, Karnataka, India during the period of May, 2015. The leaves were identified and authenticated by Dr. Kotresha K, Dept of Botany, Karnatak Science College, Dharwad; Karnataka by referring to the voucher specimen deposited in the Dept of Botany, Karnatak Science College, Dharwad, Karnataka. Fresh plant leaves material was collected and washed under running tap water, shade dried and then homogenized to coarsely powder. The powder was stored in airtight containers at -20 °C for further use for crude solvent extraction.

### Drugs and chemicals

All the solvents, chemicals and the standard drug Piperazine citrate (SD Fine Chemicals Ltd., Mumbai).

### Crude Extraction

Coarsely powdered dried leaves of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylo*n (100g each) were subjected to successive solvent extraction using Soxhlet apparatus separately. The extraction of each plant leaves material was done with different solvents in their increasing order of polarity which includes chloroform, ethyl acetate, methanol, ethanol and distilled water. Each time the plant material was dried and later extracted with next high polar solvents (following the strategy of extraction in series of increasing the solvent polarity). All extracts were concentrated in Buchi rotary evaporator, followed by removal of traces of solvent by using desiccator.

### Test organism

Indian adult earthworms (*Pheretima posthuma*) collected from the University of Agriculture Sciences, Dharwad, India. The earthworms were maintained under normal vermicomposting medium with adequate supply of nourishment and water. Before the initiation of experiment the earthworms were washed with normal saline. Adult earthworms of approximately 4 cm in length and 0.2-0.3 cm in width were used for the experiment. This organism was selected model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.<sup>22, 23</sup>

### Extract preparation for experiment

The porously powdered plants material was used for extract preparation. After extraction, the crude extract was stored in desiccator until further use. Each solvent extracts and standard drug Piperazine citrate were dis-

solved in 0.5% DMSO in normal saline (v/v). Whereas, the crude aqueous extract of all plants was directly dissolved in normal saline and used for evaluation for anthelmintic activity.

### Anthelmintic activity

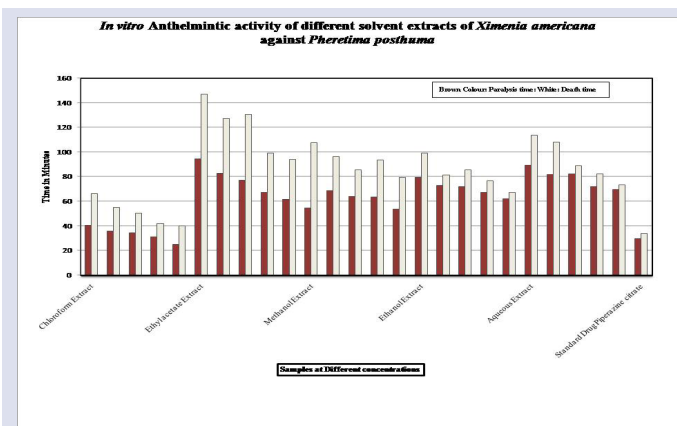
The anthelmintic activity of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylo*n extracts was evaluated by the following the method of Dash *et al.*<sup>24</sup> For each plant Twenty seven groups of animals with three earthworms in each groups, each earthworm were separate released into 20 ml of desired formulation in normal saline, Group 1 earthworm were released in 20 ml normal saline in a clean Petri plate. Group 2, 3, 4, 5, 6 earthworms were released in 20 ml normal saline containing 50, 100, 150, 200 and 250 mg/ml of chloroform extract respectively. Similarly, group 7, 8, 9, 10, 11 earthworms were released in 20 ml normal saline containing 50, 100, 150, 200 and 250 mg/ml of ethyl acetate extract respectively. Same thing will be followed for methanol, ethanol and aqueous extracts for each plant till reach of group 26. Group 27 earthworms were released in 20 ml normal saline containing standard drug piperazine citrate (100 mg/ml). Earthworms were observed; the time taken for paralysis and the time taken for death was monitored and documented in minutes. Paralysis time was analyzed based on the behavior of the earthworm with no revival body state in normal saline medium. Death was concluded based on total loss of motility with faded body color.<sup>25</sup>

### Statistical analysis

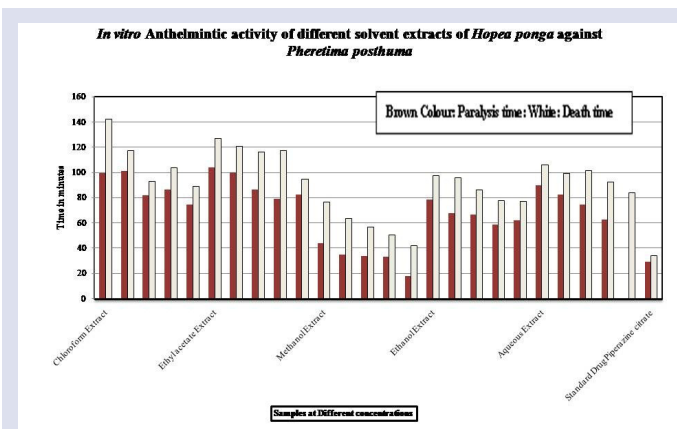
All experiments were performed in triplicates (n=3) and the data are presented as the mean  $\pm$  standard error. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software 20 Version (IBM). The significance of differences was defined at the  $p < 0.05$  and  $p < 0.01$  level.

## RESULTS AND DISCUSSION

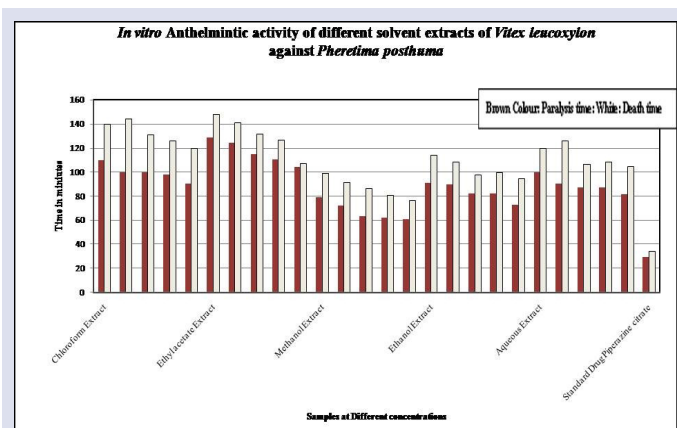
Helminthes are classified as eukaryotic endoparasites because they live inside the body, unlike parasites like lice and fleas that live outside their host. Most diseases caused by helminthes are of chronic in nature they probably cause more morbidity and greater economic and social deprivation among humans and animals. Especially in the India incidence of these diseases is at high rate especially during wet seasons with high as 100% incidence. In the India many anthelmintic drugs are available in market but these are of high cost and limited effective control over parasitic infections.<sup>26, 27</sup> Plant products are frequently considered to be less toxic and significantly free from side effects than synthetic ones.<sup>28</sup> In the present study the concentrated and dried extracts *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylo*n were evaluated for *in vitro* anthelmintic activity by varying the concentration (50-250mg/20ml) with using Indian earth worm (*Pheretima posthuma*) as animal model. In case of *Ximenia americana* many investigations have validated the use of roots in the treatment of leprosy, syphilis, dysentery, and wounds. The stem bark has been reported to have anti-trypanosomal activity. The root bark and leaf of *Ximenia americana* is used as herbal medication for the cure of many ailments by Northern part of Nigeria.<sup>29</sup> In our previous studies the aqueous and methanolic leaf extracts of *X. americana* showed significant antioxidant and anti-inflammatory activities.<sup>30</sup> In the present study among different solvent extracts of *Ximenia americana* chloroform extract exhibited highest anthelmintic activity with paralysis time  $24.66 \pm 1.20185$  (min) and death time of  $39.66 \pm 0.88192$  (min) and it was compared with standard drug Piperazine citrate (shown in Figure 1). The anthelmintic activity of remaining extracts was depicted in **Table 1**. In case of *Hopea ponga* and *Vitex leucoxylo*n among five solvent extracts methanol extract of *Hopea ponga* showed very good and significant anthelmintic activity on comparison with other tested extracts with paralysis time



**Figure 1:** *In vitro* Anthelmintic activity of different solvent extracts of *Ximenia americana* against *Pheretima posthuma*



**Figure 2:** *In vitro* Anthelmintic activity of different solvent extracts of *Hopea ponga* against *Pheretima posthuma*



**Figure 3:** *In vitro* Anthelmintic activity of different solvent extracts of *Vitex leucoxylo*n against *Pheretima posthuma*

18.33 ± 1.45297 (min) and death time of 42.00 ± 2.30940 at 250mg/20ml concentration and other remaining extracts not shown promising activity and the results are shown in Table 2 and Figure 2 and the results of tested extracts of *Vitex leucoxylo*n are shown in Table 3 and Figure 3. Even in many previous studies methanol extract of these both plants possess several biological activities i.e. methanolic extract of seed wings of *Hopea ponga* exhibits antioxidant and antibacterial activity.<sup>31</sup> Even the root and bark of *V.leucoxylo*n are reported to use as astringent and febrifuge.<sup>32</sup> Many hepatoprotective agents were isolated from leaves and bark

of *V.leucoxylo*n which includes β-sitosterol, vitexin, isovitexin and aucubin.<sup>33</sup> The results indicate that All medicinal plants and their extracts cannot exhibit anthelmintic activity in the present study also among three plants with twenty five solvent extracts only methanol extract of *Hopea ponga* and chloroform extract of *Ximenia americana* shown appreciable anthelmintic activity in performed *in-vitro* assay where as other tested extracts showed the least anthelmintic activity. These results correlating with the recent work has been done on anthelmintic activity of *Artabotrys hexapetalus* (Linn.f) and *Kalanchoe pinnata*.<sup>34, 35</sup>

## CONCLUSION

In the present study in performed *in-vitro* method for anthelmintic activity shows that all the tested extracts of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylo*n showed anthelmintic activities but among tested extracts methanol extract of *Hopea ponga* and chloroform extract of *Ximenia americana* exhibited higher anthelmintic activity over all extracts with good timing for both paralysis and Death time. At the concentration 250mg/20ml Chloroform extract of *Ximenia americana* taken 24.66 min to cause paralysis in *Pheretima posthuma* whereas methanol extract of *Hopea ponga* taken 18.33 min but standard drug Piperazine citrate taken 29.33 min at 100mg/20ml concentration. In case of causing death in *Pheretima posthuma* both chloroform extract of *Ximenia americana* and methanol extract of *Hopea ponga* taken almost nearest timing i.e. 39.66 min and 42 min respectively. Based on the present study results it can be used for the development of new pharmaceutical drugs for treatment and curing of Helminthiasis and also this study shows that these extracts offer a safe method or supplement treatment strategy to control Helminthiasis. However further comprehensive chemical and pharmacological investigation should be carried out to isolate the active compounds and appropriate elucidation of its mechanism of action and it helps in the development of new pharmaceuticals to treat Helminthiasis.

## ACKNOWLEDGEMENT

The authors would like to thank Department of Biotechnology and Microbiology, Karnataka University, Dharwad, Karnataka, India for providing necessary facilities and laboratory for doing this research work.

## CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication.

## ABBREVIATIONS USED

‰: Percentage; g: Gram; mg: Milligram; ml: Milliliter; min: Minutes; cm: Centimeter

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## PICTORIAL ABSTRACT



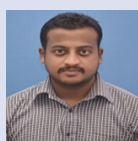
## SUMMARY

- *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylo*n were collected from Anashi forest range of Western Ghats, Uttara Kannada District, Karnataka, India and used in the traditional medicine.
- Different solvent extracts of *Ximenia americana*, *Hopea ponga* and *Vitex leucoxylo*n were subjected for *in-vitro* Anthelmintic study using Indian adult earthworm *Pheretima posthuma* as an animal model.
- *In-vitro* anthelmintic study revealed that among tested extracts methanol extract of *Hopea ponga* and chloroform extract of *Ximenia americana* exhibited higher anthelmintic activity over all extracts.
- However further study is needed for purification and isolation of active compounds from selected extracts which may serve as potential drug to treat Helminthiasis.

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**Cite this Article:** Shettar AK, Vedamurthy AB. Evaluation of *in-vitro* Anthelmintic Activity of *Ximenia Americana*, *Hopea ponga* and *Vitex leucoxylo*n. Pharmacogn J. 2017;9(3):367-71.