

## Original article

# ***In vitro* anthelmintic evaluation of common Indian Ayurvedic anthelmintic drugs: Krimimudgar Ras, Kriminol and Vidangasava against intestinal helminths**

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## ABSTRACT

Helminthiasis is one of the most common worm diseases which causes a range of adverse health problems in humans. Ayurveda is one of the most prominent and ancient systems of traditional medicines in India. Most Ayurvedic drugs used against intestinal helminths have been developed by traditional wisdom and therefore lack a proper validation through controlled studies. The aim of the present study was to scientifically validate the *in vitro* efficacy of three common Indian Ayurvedic anthelmintic drugs, viz. Krimimudgar Ras, Kriminol and Vidangasava in relation to the synthetic broad-spectrum anthelmintic drugs, praziquantel and albendazole. The *in vitro* testing of Ayurvedic anthelmintics was done against an intestinal cestode, *Raillietina* sp. and a nematode, *Syphacia obvelata*, employing 10 mg/ml, 30 mg/ml and 50 mg/ml concentrations of each medicine. The anthelmintic efficacy was judged on the basis of paralysis and mortality time of worms after exposing to these Ayurvedic drugs. Of the three tested Ayurvedic medicines, Krimimudgar ras (KR) showed the most prominent efficacy, against both the cestode and nematode parasites. At 50 mg/ml concentration, KR caused mortality of cestodes in  $7.53 \pm 0.15$  hr, and of nematodes in  $7.61 \pm 0.19$  hr. Vidangasava was found to be comparatively less effective against the tested helminth parasites. The results of this study indicate that Ayurvedic formulations do possess significant anthelmintic effects, however, an evidence-based research is required to validate all currently used Ayurvedic anthelmintics, using proper controlled studies.

**Keywords** Ayurvedic drugs, Helminthiasis, *In vitro*, Krimimudgar ras, Kriminol, Vidangasava

## INTRODUCTION

Soil-transmitted helminths (STH) or intestinal helminths are one of the most common human infections in the world. STH mainly include infections by roundworm, *Ascaris lumbricoides*, whipworm, *Trichuris trichiura* and hookworms, *Ancylostoma duodenale* and *Necator americanus*. It is estimated that about 1.4 billion people are infected with STH worldwide (Dunn et al., 2016). Currently, the community-based control of STHs is based on mass drug administration by two synthetic anthelmintics, albendazole and mebendazole. However, these drugs have been reported to have some limitations, such as toxic effects to their users and also a danger of drug resistance (Lobo et al., 2011; Kattula et al., 2014). India has a rich heritage of traditional systems of medicine, which have gained a wide acceptance in the recent years (Jabbar et al., 2007). Of all the systems of traditional medicine in India, Ayurveda is recognized as one of the most prominent and ancient systems of traditional medicine. Now-a-days, Ayurveda has received an extensive popularity among the Indian people (Dwivedi et al., 2012). Ayurvedic medicines are a collection of therapeutic entities which work

together to produce maximum efficacy with minimum side effects and are broadly classified as Rasausadhi (herbomineral formulations) and Kastausadhi (herbal formulations) (Bose et al., 2012; Barik et al., 2015). Some liquid dosage forms are also popular in Ayurvedic medicines, and they are known as Asava and Arishta. These medicines are considered superior to other dosage forms, because they have a long shelf life, besides quick to absorb and easily palatable (Das et al., 2017).

In general, most Ayurvedic medicines in India are legally manufactured under the license formulae from the natural raw materials. In addition, some Ayurvedic formulations are also manufactured as patented medicines, where their know-how is developed by product manufacturing companies based on their own expertise (Abdurahiman, 2004; Anonymous, 2014; Katoch et al., 2017; Das et al., 2017). In spite of a deep faith and a growing popularity of Ayurvedic drugs in India, information about majority of anthelmintic Ayurvedic formulations related to their efficacy and/or potential toxicity is not available in the public domain or in the form of scientific publications.

In Ayurveda, 'Krimi roga' (i.e. worm infestation) is considered one of the most common diseases in paediatric practice, and as such, includes parasitic as well as helminthic infections (Singh and Verma, 2013). At present, some Ayurvedic anthelmintics, viz. Krimighna dashemani, Vidangadi churna, Krimikuthar ras, Krumina tablet, etc. are being used for such treatments. The formulation Krimighna dashemani is composed of *Mucuna pruriens*, *Ferula asafoetida* and *Schrebera swietenoides*. Whereas, another Ayurvedic drug, Vidangadi churna is composed of *Embelia ribes*, *Hordeum vulgare*,

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*Mallotus philippinensis*, *Terminalia chebula* and rock salt. Likewise, Krimikuthar ras is comprised of *Zingiber officinale*, *Piper nigrum*, *P. longum*, *T. chebula*, *Acorus calamus*, *E. ribes*, *Commiphora mukul*, *Curcuma longa*, *Allium sativum*, *Acacia catechu*, *Ferula foetida* and sulphur as major ingredients. Also, a tablet called Krumina, consists of *E. ribes*, *A. calamus*, *Caesalpinia crista*, *Butea monosperma*, *Operculina turpethum*. Some attempts have been made to scientifically validate the potential anthelmintic efficacy of these Ayurvedic medicines, using the common Indian earthworm, *Pheretima posthuma*, as a test model (Nirmal et al., 2008; Mohandas et al., 2013; Sayyad et al., 2014).

In the present study, we conducted extensive surveys in the local markets of Shillong (Meghalaya) and Guwahati (Assam) towns of India, as well as also made a few personal interviews with the local Ayurvedic practitioners, to find out the most commonly prescribed Ayurvedic anthelmintics in this region. This study revealed that KR, Kriminol and Vidang-asava/Birangasav are some of the commonly used or prescribed Ayurvedic anthelmintics in this area. A literature survey revealed that no controlled scientific studies have been done to validate the anthelmintic potentials of these Ayurvedic anthelmintics. Therefore, keeping in view the above facts, the present study was carried out which was aimed at investigating the *in vitro* anthelmintic efficacy of KR, Kriminol and Vidangasava against an intestinal cestode, *Raillietina* sp. and a nematode parasite, *Syphacia obvelata*.

## MATERIALS AND METHODS

### Chemicals and Formulations

Systematic surveys were conducted during April to December 2016 in Shillong and Guwahati towns, India. Based on this survey, three formulations, KR (Dabur), Kriminol (Vyas) and Vidangasava/Birangasav (Assam Ayurvedic Products) were selected for this study. These medicines were purchased from local markets in Shillong. Praziquantel (PZQ) (Sigma) and Albendazole (ABZ) (Burnet Pharmaceuticals, Pvt. Ltd) were used as reference synthetic drugs. KR is a product of Dabur India in tablet form which is used as a vermifuge. The dosage varies from 125 mg to 375 mg, i.e. 1 to 3 tablets. Kriminol (syrup) is a product of Vyas Pharmaceuticals, Indore and is claimed to be useful in all types of intestinal worm infections. The dose prescribed is 2 tsp. Vidangasava, also syrup formulation, is a product of Assam Ayurvedic Products of ASIDC Ltd., Guwahati and is prescribed for curing worms, whose dosage is 12-24 ml, advised after food.

### Test parasites

Parasites belonging to the two representative group of helminths i.e. Platyhelminthes and Aschelminthes were selected for this study. Adult live specimens of *Raillietina* sp. (a tapeworm of fowl), were collected from the intestines of freshly slaughtered fowl in Shillong, while *S. obvelata* (a pinworm of rodents) adult worms were collected from the intestines of freshly necropsied Swiss albino mice, maintained in our laboratory.

### *In vitro* anthelmintic assay

The adult specimens of all collected parasites were washed several times in freshly prepared 0.9% phosphate buffered saline (PBS) at  $37 \pm 1^\circ\text{C}$ . The test worms ( $n = 6$ ) were placed in triplicates in petridishes, containing 10, 30, 50 mg/ml concentration for the solid (tablet) form and percent solutions for

the liquid dosage forms, i. e. 0.1, 0.3, 0.5 ml, in a calculated amount of PBS. Dosages were selected based on previous works of related studies (Challam et al., 2012; Thooyavan et al., 2018). PZQ and ABZ were used as reference drugs, at 1 mg/ml and 5 mg/ml concentrations, for tapeworm and pinworm parasites, respectively (Gogoi and Yadav, 2016). A separate set of test parasites, maintained in a petridish containing only PBS, served as control. After addition of test drugs, the worms were observed regularly by naked eyes for signs of their paralysis and mortality. Paralysis was confirmed when the parasites showed no signs of physical motility on exposure to slightly warm PBS ( $37 \pm 1^\circ\text{C}$ ) and also by gentle stimulation by a soft brush. Mortality was confirmed when the parasites transferred to a warmer PBS ( $40 \pm 1^\circ\text{C}$ ) showed no physical movements. The time taken for paralysis and mortality of parasites was recorded (Vijaya and Yadav, 2016).

### Statistical analysis

All data are represented as mean  $\pm$  standard errors of the mean (mean  $\pm$  SEM). Origin Pro version 8.0 SR6 was used for graphical representation. All data were analysed using one-way ANOVA followed by Tukey post-hoc test and  $p < 0.001$  was considered to be statistically significant.

## RESULTS

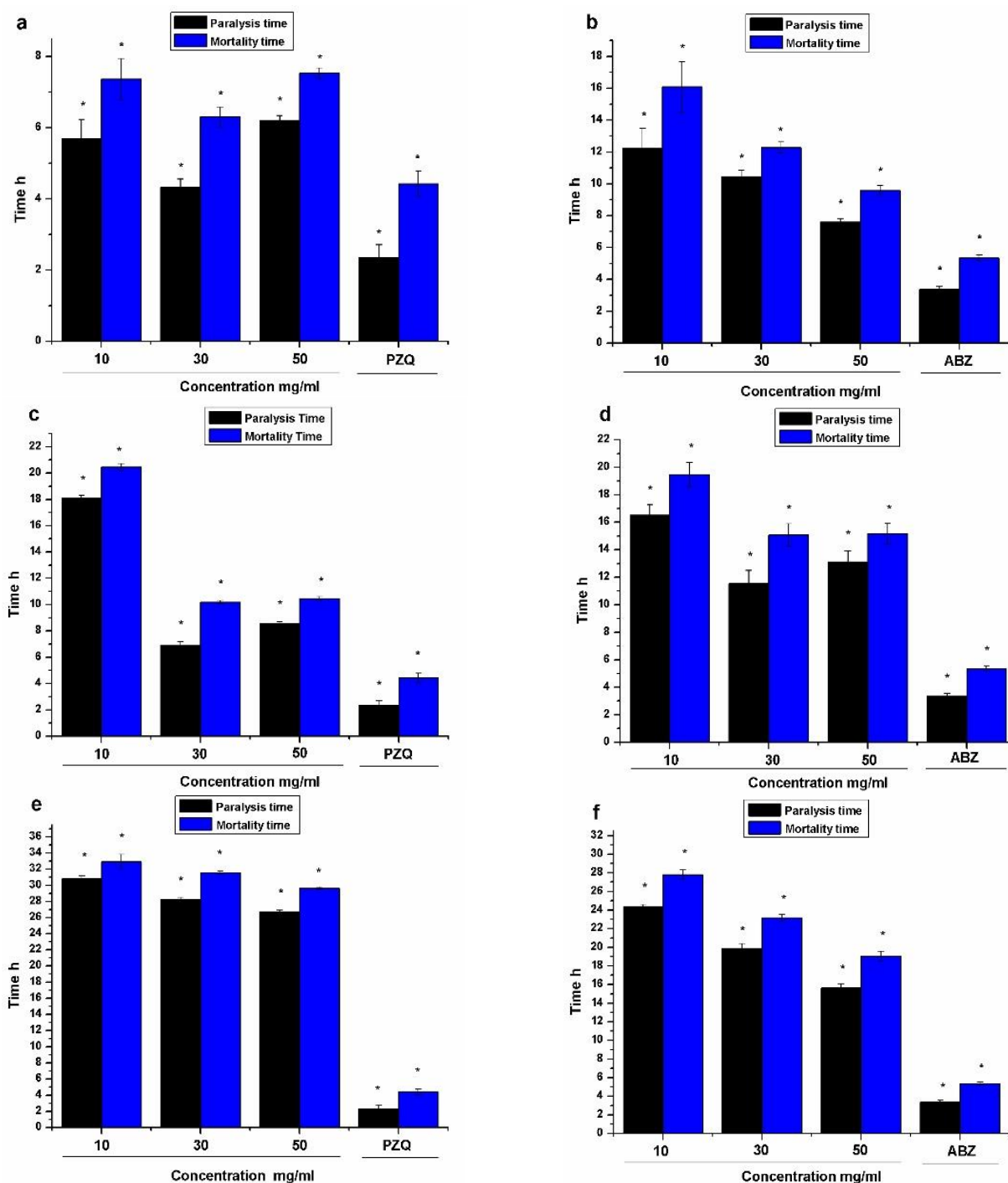
The effects of different concentrations of selected Ayurvedic formulations, as well as of reference synthetic drugs, on test parasites are shown in Fig. 1. The efficacy of all Ayurvedic formulations was observed to be dose-dependent (Fig. 1). Of the three tested drugs, Krimimudgar Ras (KR) showed the most prominent efficacy, against both the cestode, *Raillietina* sp. and the nematode, *S. obvelata* parasites. KR, at 50 mg/ml concentration, caused the mortality of *Raillietina* worms in as early as in  $7.53 \pm 0.15$  hr. This drug was also equally effective against the nematode parasite and caused their mortality in  $9.59 \pm 0.30$  hr. The second best efficacy was observed with Kriminol, which showed the mortality of cestode and nematode parasites in  $7.53 \pm 0.15$  hr and  $15.16 \pm 0.75$  hr, respectively. Notably, the third tested Ayurvedic drug, Vidangasava did not show a good efficacy. Vidangasava took  $29.64 \pm 0.15$  hr and  $19.04 \pm 0.54$  hr to cause the mortality of cestode and nematode parasites, respectively. In all the above experiments, the reference synthetic drugs, PZQ and ABZ, showed mortality of *Raillietina* sp. and *S. obvelata* in  $4.42 \pm 0.35$  and  $5.36 \pm 0.16$ , respectively (Fig. 1).

## DISCUSSION

Although a few workers have attempted to validate the anthelmintic potential of some Ayurvedic medicines, however, most of these studies seem to have one or other limitations. For example, Reddy and Seetharam (2009), Verma et al. (2011) and Dwivedi et al. (2009), in their *in vitro* study to validate the anthelmintic efficacy of some polyherbal Ayurvedic anthelmintic formulations, used the common Indian earthworms as a test model. It may however be noted here that except some morphological similarities, earthworms as such are free-living organisms and therefore they do not possess any anatomical or physiological resemblance with intestinal helminths. Therefore, the findings of these studies may not as such be applicable for intestinal helminths.

The results of the present study revealed a dose-dependent efficacy of all the tested Ayurvedic formulations. Interestingly, KR showed the best efficacy against both the parasites, *Raillietina* sp. and *S. obvelata* in comparison to control worms. The activity of KR was recorded to be almost as good as those of synthetic drugs. Also, Kriminol showed the second best

anthelmintic efficacy. However, the third formulation, i.e. Vidangasava showed a very poor anthelmintic efficacy as compared to KR and Kriminol formulations. The differences in the efficacies of tested medicines may be explained on the basis of nature of their ingredient.



**Fig. 1.** *In vitro* anthelmintic effects of common Ayurvedic formulations and reference drugs (PZQ and ABZ) against *Raillietina* sp. and *Syphacia obvelata*: KR against (A) *Raillietina* sp. (B) *S. obvelata*, Kriminol against (C) *Raillietina* sp. (D) *S. obvelata*, Vidangasava against (E) *Raillietina* sp. (F) *S. obvelata*. The physical activity of worms maintained in the control medium was observed till  $43.26 \pm 1.01$  hr for *Raillietina* sp. and for  $43.85 \pm 1.25$  hr for *S. obvelata*. All data are represented as mean  $\pm$  SEM; Significant at  $p < 0.001$ .

Although all tested formulations contain *E. ribes* as their common ingredient, but the drugs, i.e. KR and Kriminol, which showed a significant efficacy in this study, also contained two unique ingredients, i.e. *Butea monosperma* and *Apium leptophyllum* that is not found in Vidangasava, which showed poor anthelmintic effects. Further, the most significant efficacy of KR in this study also seems due to presence *Melia azedarach* as an exclusive ingredient of this formulation. Besides, KR also possesses black sulphide of mercury (kajjali) which is not found in other two test formulations. In Ayurveda, kajjali has been reported to play a major role in the treatment of various other common diseases (Srinivasulu et al., 2012; Bhagyalakshmi et al., 2016). It may therefore be suggested here that the unique ingredients present in KR possibly work together to produce a synergistic effect. Nirmal et al. (2008) investigated the anthelmintic potentials of some Ayurvedic formulations viz. Krimikuthar ras, Kumariasava, Bhallatkasava and Vidangarishta. The results of this study showed that some ingredients of these drugs, namely *Zingiber officinale*, *Piper longum*, *Commiphora mukul*, *Curcuma longa*, *Allium sativum*, *Terminalia chebula*, *Ferula foetida* and sulphur, when present together or in different combinations, show different anthelmintic effects. Similarly, in another report by Anwikar and Bhitre (2010), the combination of extracts of *Solanum xanthocarpum* and *Cassia fistula* revealed better anti-inflammatory effects than individual tested extracts. It therefore appears that a synergistic effect may be due to the correct combination of different ingredients present in the efficacious formulation. The phytochemistry of herbs present in efficacious formulations have also been studied previously and these plants have been reported to contain sterols, phenols, glycosides, flavonoids, saponins as their common constituents. The phytoconstituents such as phenols, terpenoids, glycosides, saponins have been reported to possess significant effects on parasites by interfering with either their energy generation mechanism or causing their membrane disruption, leading thereby to their paralysis and/or mortality (Bhandari and Ansari, 2008; Rajput et al., 2011; Thite et al., 2013; Bauri et al., 2015).

In other related studies, Abhilasha and Dubey (2017) reported that when a combination of Vidanga beeja churna and Kampillaka phalraja churna is administered to patients infested with roundworms, the results are promising. Danga et al. (2014) employed an Ayurvedic formulation, Khadiradi Kashaya for the management of worms and it also showed very appreciable results. Further, as reported by Singh et al. (2014), a patient with *Hymenolepis nana* infection was administered with another Ayurvedic medicine, Krimighna Kashaya vati (Krimighna dashemani) for a period of 28 days and the results were encouraging. However, as of now not many controlled studies or clinical trials have been undertaken for a majority of anthelmintic drugs. Nevertheless, as evident from our study, it may be said that Ayurveda drugs and formulations may play a key role in the treatment of helminthiasis. However, a proper scientific validation is necessary for all Ayurvedic anthelmintics for their systematic use and also for a wider acceptance of these medicines outside India.

## CONCLUSION

To sum up, the findings of this study suggest that Krimimudgar ras possesses maximum efficacy against both, the cestode and nematode parasites, which is followed by Kriminol. However, Vidangasava was found comparatively less effective against helminth parasites. It may therefore be said that not all

commonly used Indian Ayurvedic medicines possess satisfactory degree of anthelmintic effects. Therefore, all currently used anthelmintic Ayurvedic medicines should be evaluated using proper controlled studies. A proper validation of Ayurvedic drugs may not only lead to further growth and popularization of these drugs in India but may also promote these drugs outside India.

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## CONFLICT OF INTEREST

The authors report no conflicts of interest.

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