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R Edith
Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

BR Latha
Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

M Balagangatharathilagar
Department of Veterinary Clinical Medicine, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Corresponding Author:
M Balagangatharathilagar
Department of Veterinary Clinical Medicine, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Evaluation of anthelmintic efficacy of *Pithecellobium dulce, Momordica charantia* and *Carica papaya* leaf extracts on egg hatching and larval development of *Haemonchus contortus*

R Edith, BR Latha and M Balagangatharathilagar

Abstract
Screening of plants for anthelmintic property is a promising alternative for routine chemical anthelmintics drugs to reduce the problem of anthelmintic resistance and to avoid drug residues in meat and milk products. *Haemonchus contortus*, the stomach worm of sheep, causing severe economic loss to the Indian farmers in terms of decreased body weight gain and reproduction. The leaves of *Pithecellobium dulce* (Manila tamarind), *Memordica charantia* (Bitter guard) and *Carica papaya* (Papaya) In the present the aqueous leaf extracts of *P. dulce*, *M. charantia* and *C. papaya* were screened for their anthelmintic effects by *in-vitro* Egg hatch assay and Larval development inhibition assay at 0.5, 1, 2, and 5 percent concentrations in comparison with thiabendazole. *P. dulce* leaf aqueous extracts showed inhibitory effect on egg hatching though there was initiation of embryonation at first 24 hrs of incubation. However, no degeneration of egg shell was observed. *M. charantia* leaf aqueous extracts showed hatching and immediate mortality of all hatched out larvae at all concentrations. Aqueous *C. papaya* leaf extracts of showed inhibition of egg hatching and degeneration of eggs with varying percentages at all concentrations.

Keywords: *Pithecellobium dulce, Momordica charantia, Carica papaya, Haemonchus contortus,* leaf extract and anthelmintic activity

Introduction
Sheep farming plays an important role in the socioeconomic upliftment of small and marginal farmers of rural India. Sheep are one of the earliest animals to be domesticated for agricultural purpose. Sheep husbandry is practiced throughout the majority of the inhabited world, and has been fundamental to many civilizations.

India stands in third place among the other countries in sheep raring (74.26 million). Livestock sector contributes 4.11% GDP and 25.6% of total Agricultural GDP**.** Sheep also play a major role in many local economics and sheep flocks may be a part of agriculture rather than a system of trade. Sheep rearing provides livelihood supports in term of income and employment to millions of land less small pastoralist. Sheep manure is an important source of organic fertilizer and contributes to a part of earning of a farmer, particularly in India. Sheep skin is likewise used as making clothes, footwear, rugs and other products. Byproducts from the slaughter of sheep are also for value. Sheep intestine can be formed into sausage casing and lamb intestine has been formed into surgical sutures.

Sheep are prone to many diseases including parasitic disease. Intestinal parasitic infections are one of the major problems in the world, especially tropical and subtropical countries. Sheep are infected with several species of gastrointestinal (GI) nematodes that evolved from their free living forms due to lack of oxygen. Many species of parasites are seen in sheep and goat and usually include *Haemonchus* sp., *Ostertagia* sp., *Tichuris* sp., *Moniezia* sp., etc. Among them, *Haemonchus contortus*, are highly evolved and are major productions limiting helminthic infection in sheep and goats. It is highly pathogenic, blood feeding nematode of small ruminants and a significant cause of mortalities worldwide. Disease occurrence is more common in tropical and subtropical countries due to hot climatic conditions. *Haemonchosis* can occur in all age groups. Clinical *Haemonchosis* is mainly classified into three types depending upon intensity of worm burden that is hyper acute, acute, chronic form. Acute haemonchosis is most common in India and is characterized by anaemia, hypoprotenemia and development of bottle jaw condition. 30-40% mortality has been reported in lambs due to

haemonchosis if no timely treatment with proper anthelmintic is undertaken [1].

At present treatment and control of helminth infections carried out using anthelmintic drugs. A lot of money is spent annually worldwide for controlling parasitic nematodes using anthelmintic drugs. However, now a day there is an emergence of anthelmintic resistance which has influenced on the success of conventional anthelmintic drugs for the control of GI nematodes. Frequent use, increased dosage and increased application rate are well correlated with declining effectiveness of synthetic anthelmintics. The emergence of anthelmintic resistance and increasing public awareness over the drug residues in animal products has necessitated the alternate, sustainable, economic and ecofriendly strategies like medicinal plants as anthelmintics. More over there is an increasing awareness among people for chemical free food and animal products usage. Hence, new alternative to this chemical anthelmintic is need of the hour for the treatment and control of helminthic infections medicinal plants may act as a alternative ecofriendly, sustainable alternative instead of chemical anthelmintic with no side effects for helmintic control. Plants play an important role in the health care of the about 80 percent of the population and is estimated that more than half of the drugs under clinical use at present own their origin to plants [2].

Several plants have been evaluated for their anthelmintic activity against *H. contortus viz.*, *Syzygium aromaticum* and *Melia dubia* with 50% reduction in egg hatching and 100% effect on motility of the worms [3]. *Iris kashmiriana* showed 100% mortality in adult worms and 70% of reduction in faecal egg count [2]. *Maytenus senegalensisi* showed mortality in adult worms and 37.77% inhibition of larval migration [4]. *Indigofera tinctoria* showed 30-47% reduction in faecal egg count [5]. *Momordica charantia* leaf extracts showed 80-85% inhibition of egg hatching of *Fasciola hepatica*.

Pithecellobium dulce (Manila tamarind) is the tree fodders fed to sheep and goats in India. It's bark and pulp are used as a traditional remedy against gum ailment, toothache, and hemorrhage. Bark extract is also used for dysentery, diarrhea, and constipation. An extract of leaves is used for gallbladder ailments and to prevent miscarriage. Its leaves cure indigestion, cleans gastro intestine tract and kill intestine germs. Seeds pods contain a sweet and sour pulp, when grounds are used to cleanse ulcers. Prevent cancer, eliminates pigmentation, cures acne and pimples, removes dark spot, slow down aging, natural skin moisturizer, lightens skin, prevent hair loss [6, 7].

Momordica charantia (Bitter guard), is also known as bitter melon grows in tropical and subtropical region. Leaves are used for the treatment of jaundice and other liver disease and to cure ulcers and burns. It also used for the treatment of high blood pressure, infection, malaria, fever, cold, gout, kidney stones, peptic ulcers, dysentery and worm infection. It acts as a blood purifier, helps in weight loss, improves immunity. If is widely used for diabetes, fight acne [8].

Carica papaya (papaya) leaves are commonly used against dengue; being considered as one of the healthiest fruits, papaya helps in curing many diseases not only pulp but its leaves contain many healing properties. Papaya is also known as pawpaw [9].

The present study was aimed to evaluate anthelmintic activity of aqueous leaf extracts of commonly available plants of India *viz. Pithecellobium dulce*, *Momordica charantia* and *Carica papaya* on *Haemonchus contortus*.

Materials and Methods

Collection of *H. contortus*: Sheep abomasum along with its contents was collected from slaughter house, Perambur Chennai were brought to the laboratory. The content with live worm was transport to a Petri dish containing normal saline (Fig. 1). The adult male and female *H. contortus* worms were separated, washed twice in the normal saline and collected. These collected worms were used for further studies.

Harvesting of *H. contortus* eggs: The female worms were incubated in normal saline at 37 °C for 1 hour to release eggs naturally and male worm were discarded (Fig.2). After incubation, the normal saline was collected in tubes and centrifuged at 2000 rpm for 5 minutes to sediment the eggs. The supernatant was poured off and the sediment was examined for the eggs. The concentration of the eggs was adjusted to 35-40 eggs per 500 µl. These eggs were immediately used for egg hatch assay (Fig.3).

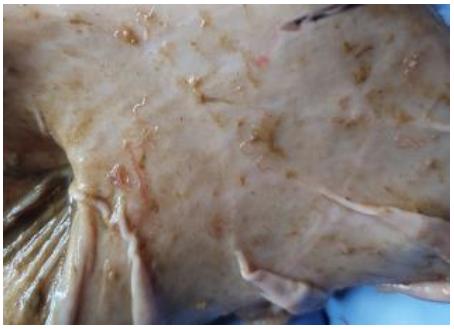

Fig 1: Abomasum of sheep with *Haemonchus contortus* worms

Fig 2: Adult female *Haemonchus contortus* worms in Normal saline incubated at 37 °C

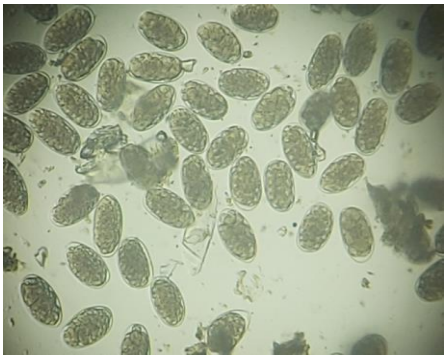

Fig 3: Harvested eggs of *H. contortus*

Collection of plant materials: *Pithecellobium dulce* (Manila Tamarind), *Carica papaya* (papaya) and *Momardica charantia* (Bitter guard) were collected from the agricultural field. The mature plant leaves were collected in polythene bags and transport to laboratory. All fresh leaves were washed in water, drained and were cut into small pieces. The plant materials were shade dried till it become brittle. The dried leaves were powered using an electric stainless steel blender. The powered plant materials were stored in an airtight container.

Preparation of plant extracts: Aqueous extract was prepared by dissolving 15g of powered each plant material in 150 ml of distilled water. It was allowed to macerate for 24hrs at room temperature. Then the percolation proceeds. It was filtered using what man no.1filter paper. The filtrate was evaporated by keeping in hot air oven at 32 °C. The final crude aqueous extracts was scrapped and transferred to an airtight container and stored for further studies (Fig.4). The above procedure was repeated for all the three plant samples.

Shade dried leaves, powdered leaves and dried aqueous extract of *Pithecellobium dulce*	Shade dried leaves, powdered leaves and dried aqueous extract of *Carica papaya*	Shade dried leaves, powdered leaves and dried aqueous extract of *Momordica charantia*

Fig 4: Preparation of plant extracts

Egg Hatch Assay: The method described by Laurde *et al*. [10] was followed for egg hatch assay. To each well of a 48 multiwell plate, 500 µl of egg suspension containing approximately 35-40 eggs was added. 500µl of working solution of 0.1; 0.3; 0.5; 1.0;5 µl/ml Thiabendazole (TBZ), the standard drug, was added to each well. 500 µl of aqueous extract of *Pithecellobium dulce* (*kodukapuli*), *Momordica charantia* (*Bitter guard*) and *Carica papaya* (*papaya*) with a final concentration of 0.5%, 1%, 2% and 5% was added in different wells. 500µl of Dimethyl Sulfoxide (DMSO) was added to the control well. The tests were carried out with two replicates for each drug concentration plus control well. The volume in each well made up 2 ml using distilled water. The plate was incubated at 37 °C for 48 hrs. After incubation, one drop of Lugol's Iodine was added to stop further embryonation of eggs. The mean number of eggs and larvae at each concentration of TBZ was counted under a binocular light microscope and percentage of hatch was derived using the following formula to know the resistance status.

$$\text{Percentage hatch} = \frac{\text{Numbering of larvae hatched}}{\text{Total number of eggs added}} \text{ X100}$$

The percentage hatch was taken as percentage of resistance as the larvae survived anthelminthic treatment. Similarly after 48hrs, numbering of eggs embryonated and unembryonated were counted under a binocular light microscope. Percentage of unembryonated eggs was calculated to know the susceptibility of the extracts used.

$$\text{Percentage efficacy} = \frac{\text{Total number of eggs added - No. of larvae hatch}}{\text{Total number of egg added)}} \text{X 100}$$

Results

In the present study, the anthelmintic efficacy commonly available plant leaves *viz.*, *Pithecellobium dulce* (Manila Tamarind or Madras thorn), *Momordica charantia* (bitter guard), and *Carica papaya* (Papaya) against the nematodes parasite *Haemonchus contortus* was evaluated by egg hatch assay in comparison with standard anthelmintic thiabendazole.

The *in vitro* anthelmintic efficacy of the aqueous leaf of the above mentioned plants extracts at 0.5%, 1%, 2%, 5% concentrations were used in this study. Thiabendazole, the standard anthelmintic was used at 0.1µg/ml, 0.3µg/ml, 0.5µg/ml, 1.0µg/ml concentrations. In the 48 well culture plates, eggs of *Haemonchus contortus* were added long with the plant extracts at different concentrations and were incubated at 37^0 C and were observed at 24hrs interval for the changes in development of *Haemonchus contortus* eggs.

All concentrations of *Carica papaya* aqueous leaf extracts showed inhibition of egg development and hatching. There was initial condensation of yolk material with varying percentage i.e. 70, 60, 40 and 30 at 0.5%, 1%, 2%, and 5% concentration respectively at 24hrs. However, further development was found arrested at all concentrations and at 48^{th} hr observation, degeneration of egg shell was noticed at all concentrations (Fig. 5)

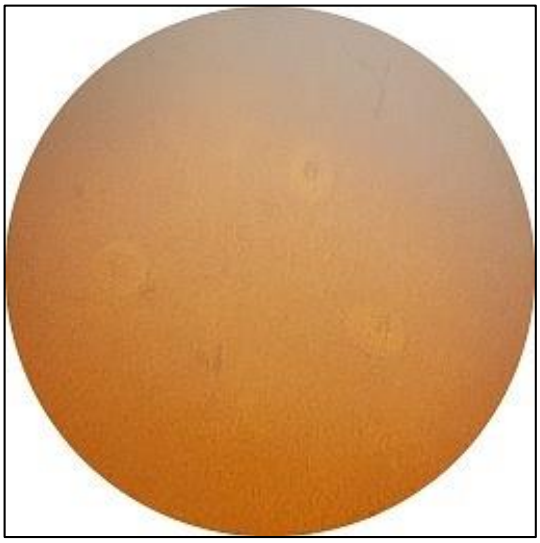

Fig 5: *Carica papaya* extracts (2%) showing degradation of eggs

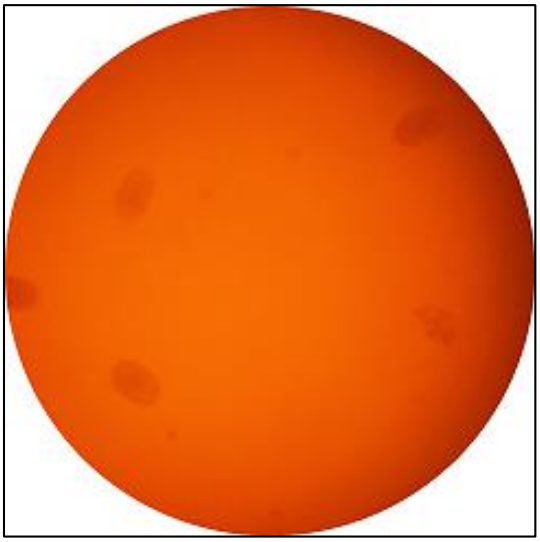

Fig 6: *Pithecellobium dulce* leaf extracts (5%) showing inhibition of egg hatching

At 24^{th} hr observation the aqueous extracts of *Pithecellobium dulce* leaves showed 60%, 70%, 75% and 80% prevention of embryonation was observed in 0.5%, 1%, 2%, and 5% concentrations respectively. After 48hrs, the embryonation was 85%, 80%, 75% and 70% percentages in 0.5%, 1%, 2% and 5% concentrations respectively. However, no further development of embryo inside the egg was observed and hence no egg hatched after 48hrs. No degeneration of egg shell was noticed after 48hrs incubation (Fig.6).

In aqueous extracts of *Momordica charantia* leaves 80%, 85%, 90%, 95% egg hatching was observed in 0.5%, 1%, 2% and 5% concentrations respectively at 24hrs itself but no larva was alive after hatching. At 48hrs 100%, 95%, 92% and 90% egg hatching was observed and not even a single larva was alive at 0.5%,1%, 2% and 5% concentrations respectively of aqueous extracts of Bitter guard leaves (Fig. 7 and Fig.8)

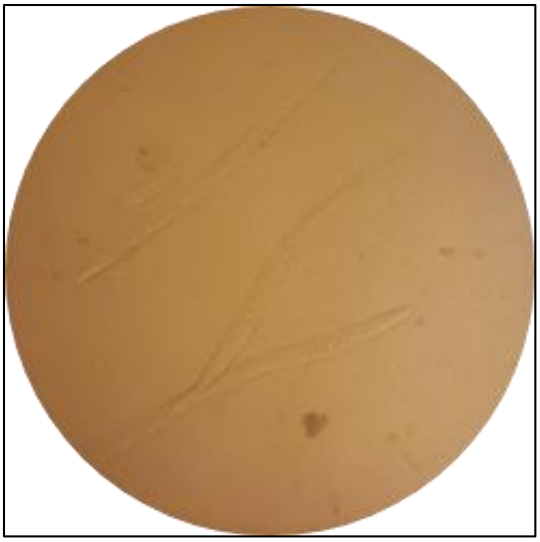

Fig 7: *Momordica charantia* extracts (2%) Showing immediate mortality

Fig 8: *Momordica charantia* extract (5%) showing immediate mortality

Discussion

Gastrointestinal nematodes infection is considered as a major constraint in the livestock sector due to the huge economic losses caused by them in terms of morbidity and mortality. Among the gastrointestinal nematodes of small ruminants *Haemonchus contortus* is a very important parasite. Many medicinal plants have been screened and used as an alternative to routine chemical anthelmintics. Screening of medicinal plants for its anthelmintic properties is mainly done by *in-vitro* analysis.

The advantage of *in-vitro* assays is that the compounds or materials to be tested are in direct contact with the different life-cycle stages of parasites. More over *in-vitro* screenings of anthelmintic agents prior to *in-vivo* testing have shown to be a rational and practical strategy since they save time and money and also minimize the number of animals necessary for the development of a new therapeutic agent [11]. In this study the egg hatch assay, *in-vitro* method was used to study the anthelmintic activity of aqueous extracts of leaves of three commonly available plants viz., *kodukapuli* (*Pithecellobium dulce*), *bitter guard* (*Momordica charantia*) and *papaya* (*Carica papaya*).

Aqueous leaf extracts of *Carica papaya* has shown inhibitory effect on egg hatching of *Haemonchus contortus* eggs. Though there was initiation of embryonation (30-40%) at 24hrs of incubation, further development was completely inhibited and no hatching of eggs was observed even at the lowest (0.5%) concentration. The leaf extracts of *Parkiabi globosa* inhibited hatching of nematodes eggs due to the saponins and tannins present in the extracts [12]. In the present study, phytochemical analysis was not carried out for the aqueous extracts. However, the egg hatch inhibitory effect observation in this study might be due to the presence phytochemicals in the extracts. The effect of saponins is due to their interaction with the cell membranes, changes in the cell wall permeability and interaction with the collagen proteins from the cuticle of nematodes. It has been reported that tannins have the capacity to bind to proteins and inactivate many mechanisms including the nutrient availability if the larvae, there by produces the anthelmintic effect [13].

At 48 hrs observation, the papaya aqueous extracts showed disintegration of the egg shell and disruption of ova in all concentration. This effect clearly indicates that the papaya leaf extracts is highly effective against nematodes by causing disintegration of ova and there by inhibition of egg hatching. The anthelmintic activity of ethanolic extracts of *Carica papaya* leaves was evaluated by using *Paramphistomum cervi* and *Haemonchus contortus* by *in vitro* method. They have reported dose dependent high effectiveness of the extracts against *Haemonchus contortus* adult worm. Our present study indicated that the anthelmintic effect of *C. papaya* leaf extracts is through degeneration of egg shell and destruction of ova.

Aqueous leaf extracts of *Momodica charantia* had shown no inhibitory effect on the egg hatching. However, there was immediate mortality of the hatched out larvae which was quite different and interesting to observe. The immediate mortality of hatched out larvae by the aqueous extracts of *M. charantia* can be considered for the control the worm load that are present in the abomasums of ruminants.

Aqueous leaf extracts of *Pithecellobium dulce* showed inhibition of egg hatching of *H. contortus* eggs. Initiation of embryo development inside the egg in the form of yolk condensation was noticed with varying percentages i.e. 30-40% concentration 50% in 1% concentration and 30-60% in 2% and 5% concentration of extracts. However, at 48hrs observation no further development was observed and there was 0% hatching at all concentrations which clearly elucidate the egg hatch inhibition effect of aqueous leaf extracts of *P. dulce*. The *P. dulce* is a very commonly available tree in rural South India and the leaves can be regularly fed to the sheep and goats to control the nematodes infections.

Conclusion

The findings of this study indicated that the aqueous leaf extracts of three plants *viz*., Papaya (*Carica papaya*), Bitter guard (*Momordica charantia*) and Manila Tamarind (*Pithecellobium dulce*) have anthelmintic activity against *H. contortus*. Among all the three aqueous leaf extracts, *Momordica charantia* (Bitter guard) was found have more potential anthelmintic activity compared to the other two plants. *P. dulce* leaf aqueous extracts showed inhibitory effect on egg hatching though there was initiation of embryonation at first 24hrs of incubation. However, no degeneration of egg shell was observed. Whereas, *M. charantia* aqueous leaf extracts showed immediate mortality of all hatched out larvae at all concentrations. Aqueous *C. papaya* leaf extracts showed inhibition of egg hatching and degeneration of eggs with varying percentages at all concentrations. The phytochemical analysis and *in-vivo* studies of these extracts are warranted for further application of this research work.

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Conflict of Interests

The authors do not have any conflict of interest

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