

Efficacy of Jack Fruit extract (*Artocarpus heterophyllus*) on *Haemonchus contortus*

ABSTRACT

Haemonchosis is the one of most important gastrointestinal parasitic infection that affects the small ruminants worldwide. The disease causes severe anaemia, stunted growth, decreased production, less weight gain and sometimes lead to mortality. Anthelmintics are commonly employed for the control of these parasites. However, inappropriate and extensive use of these drugs has contributed to the development of resistance. There is a demand for alternative control measures. Herbal anthelmintics can act as a source of affordable and alternative anthelmintic agents. In this context a study was conducted to determine the *in vitro* efficacy of ethanolic extract of Jack fruit using Adult mortality assay. Fruit portion of the plant was used for ethanolic extract. The ethanolic extract was used at various concentrations *viz.*, 10%, 30%, 50%, 70% and 90%. The test was carried out in triplicates for each concentration and fenbendazole was used as a positive control. Observation was done at every 30 minute intervals. At 10% concentration, 100% mortality was observed in 2 hours which was similar to that of positive control. Microscopic observation of the dead worms revealed the alteration in the integrity of the cuticle. Further in-depth studies are required to confirm the anthelmintic activity of the plant.

Keywords: herbal anthelmintics, jack fruit, haemonchosis, *in vitro*

1. INTRODUCTION

Livestock is one of the most essential, complex, diverse and fast growing subsectors of the agriculture in developing countries. Small ruminants play an important role in the Indian economy and provide livelihood to about two-third of the rural community. They contribute greatly to the agrarian economy, especially in areas where crop and dairy farming are not economical and play an important role in ushering livelihood security to large proportion of small

and marginal farmers as well as landless labourers. Nevertheless, sheep production is hindered by several factors such as parasitic infections, inadequate nutrition and other managerial practices.

Among the parasitic diseases of small ruminants, gastrointestinal nematode infection ranks the highest on a global index. The most commonly encountered gastrointestinal nematodes of sheep and goats include *Haemonchus contortus*, *Teladorsagia (Ostertagia) circumcincta*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Nematodirus spp.*, *Bunostomum trigonocephalum*, *Oesophagostomum columbianum*, *Cooperia curticei*, *Strongyloides papillosus*, *Trichuris ovis* and *Chabertia ovina*. *H. contortus*, is a highly pathogenic nematode parasite. Haemonchosis is one of the most important gastrointestinal parasitic infection that affects the small ruminants worldwide. The harmful effects caused by these gastrointestinal nematodes could range from weight loss in subclinical infections to lethal pathologies such as anaemia, reduction in functional gastric gland mass, severe damage to the gastric mucosa, villous atrophy etc, leading to diarrhoea and severe protein loss. In addition, parasitism could result in indirect serious consequences on metabolism such as mobilisation of proteins for immune function, reduced feed intake, loss of productivity, unthriftiness, delay in puberty, loss of meat, poor reproductive performance, increased susceptibility to other infections and death in severely infected individuals. It represents an animal welfare problem worldwide.

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Livestock production obtains considerable benefits from the use of anthelmintics in controlling livestock parasites. The annual integrated cost of the three helminth infections in 18 countries participating in COMBAR (2020) (Combatting Anthelmintic Resistance in ruminants) was estimated to be €1.8 billion [€1.0–2.7 billion] (Charlier *et al.*, 2020). Nearly 81% of this cost (€1.46 billion [€0.84–2.10 billion]) comprised of charges owing to production losses and 19% (€0.35 billion [€0.14–0.57 billion]) accredited to treatment costs. Currently, known public

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expenditure in research on controlling helminth infections is approximately 0.15% of the annual expenses for parasitic diseases (Charlier *et al.*, 2020).

For decades, anthelmintics acted as cornerstones for the control of parasites. Due to the general lack of effective antiparasitic vaccines use of anthelmintics in sheep becomes the only practical method to reduce the adverse effects of these nematode parasites. Adversely, because of their frequent use, anthelmintic resistance reports have increased around the world (reviewed by Gilleard *et al.*, 2021).

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The emergence of resistance to anthelmintics has created a necessity for the animal production sector to explore possible alternative anthelmintic options. Unlike commercial anthelmintics, potential anthelmintic alternatives should be biodegradable, have no contaminants in meat, and be highly effective. Consequently, research is exploring plants with potential anthelmintic activities used by resource-poor farmers. These plants have not been sufficiently evaluated *in vitro* and *in vivo* for their anthelmintic effects, toxicity and residual effects on the host animal(s). Hence, traditional practice needs to be improved so that active natural chemicals can be identified (Mhlongo *et al.*, 2024).

One of the feed plant that has the potential to serve as an anthelmintic is the jackfruit, as jackfruit leaves are used as an alternative forage during the dry season and they contain tannin antinutrient compounds. Tannin compounds in forages have a protective effect on the intestinal mucosa, so their consumption can reduce the population of parasites in the small intestine epithelial cells. The number of mast cells in the mucosa of the small intestine can be decreased by feeding tannins (Nguyen *et al.*, 2013). Actual cases now indicate that anthelmintics developed from tanniniferous plants can serve as potential strategic alternatives for controlling nematode infestations in small ruminants (Akkari *et al.*, 2008).

Tacbas *et al.*, 2024 in their study has suggested that the extracts of *A. heterophyllus* and *A. camansi* can be a potential alternative for anthelmintic treatment or with further research, can be utilized as a natural source and active ingredient for a bio-based anthelmintic pharmaceutical drug. Few studies have been conducted using the leaves and seeds of Jack fruit extract (*Artocarpus heterophyllus*) on *H. contortus*. But the studies using pulp portion of Jack fruit is very meagre hence, a study was carried out to know the anthelmintic efficacy of Jack fruit extract (*Artocarpus heterophyllus*) on *H. contortus*.

2. METHODOLOGY

2.1 Preparation of extract:

The jackfruit is cut into two pieces; the fruits are separated from the rinds, rags, and seeds. The isolated fruits are sliced into small pieces for better drying. The sliced jackfruits are placed in hot air oven (LABLINE, A matrix instrument technologies) at 50 °C for seven days. After a week, the dried jackfruit slices are pulverized into a coarse powder using an electric mixer and subjected to extraction. The jackfruit powder (300gm) was subjected to Soxhlet extraction using 99.99% ethanol (1:10) for 14 hours at 50°C. The obtained extract was then concentrated under reduced pressure at 50°C using rotary evaporator. The extract was further concentrated by allowing it to stand in a water bath at 50°C for 12 hours to remove trace of ethanol solvent. The dried extract was sealed in a bottle and stored in the refrigerator at 4°C until further used. Out of 1 kg pulp portion of fruit nearly 150-200ml extract was obtained. The extract was very viscid in nature and brownish in colour. In the study, the ethanolic extract was used at various concentrations viz., 10%, 30%, 50%, 70% and 90%.



Fig 1: Jackfruit extraction

2.2 Collection of adult *H. contortus*

The adult *H. contortus* worms were collected directly from the abomasum of naturally infected sheep that had been slaughtered at slaughterhouse. Following the slaughter, abomasum was opened and the fecal contents were carefully removed. The parasites embedded to the abomasal mucosa (Fig. 1) and those present in the faecal contents were collected and washed in normal saline. Later they were stored in phosphate buffered saline (PBS) (Kuchai *et al.*, 2012) for further use (Fig. 2).

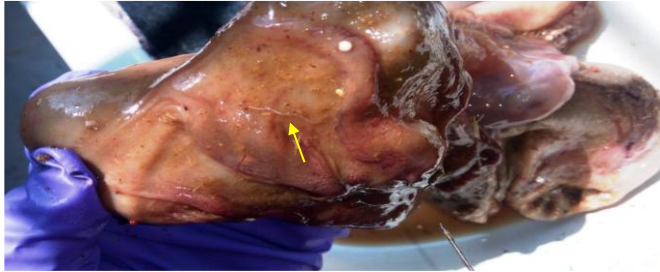


Fig. 2: *H. contortus* worm embedded to the abomasal mucosa



Fig 3. *H. contortus* worms retrieved from the abomasal contents

The harvested worms were subjected for morphological characterization. Some of the retrieved adult worms were first washed in normal saline and then mounted in Rubin's mountant and left as such until the mountant gets dried. Later the worms were identified. Morphological parameters used to study male *H. contortus* worms included body length (10 – 20 mm), cervical papillae length, spicule length, and cuticular ridges. Female *H. contortus* worms have a “barber's pole” appearance, with white ovaries and uteri twisted around a red blood-filled intestine, while males are uniformly reddish-brown when observed fresh. Female *H. contortus* species can be differentiated based on body length (18 – 30 mm), cervical papillae length, number of cuticular ridges, and vulva flap morphology represent the main criteria for identifying and differentiating female parasite species. Additionally, it has a tooth or lancet in its poorly developed oral cavity, which helps perforate the gastric mucosa and suck blood (Gareh *et al.*, 2021).

2.3 Adult mortality assay

The adult worms were divided into 5 groups of 10 worms per group (Alemu *et al.*, 2014). The test was carried out in triplicates for each concentration. Distilled water was used as a negative control and fenbendazole as positive control. Each treatment consisted of three trials. Each group of worms were immersed in the ethanolic extract of fruit portion of Jack fruit. Observation was done at every 30 minute intervals upto 4 hrs.

The inhibition of motility and/or mortality of the worms exposed to the above concentrations was used as an indicator for anthelmintic activity. Finally, the extracts and fenbendazole were washed and the worms were re-suspended in lukewarm fresh PBS for 30 min to observe and test the revival of motility. Death of the worms was ascertained by the absence of motility for observation period of 5–6 s.

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3. RESULTS AND DISCUSSION

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In the present study, at a low concentration of 10%, 100% mortality of worms was observed in 2 hours which was similar to that of positive control. None of the worms showed the revival of motility after they were placed in lukewarm PBS for 30 min. The pulp portion of Jackfruit showed effect on the morphometry of *H. contortus*. Microscopic observation of the dead worms revealed the alteration in the integrity of the cuticle.

Al- Rofaai *et al.* (2012) suggested that the mechanism of saponin on the cell membrane contributes to the disintegration of the parasite teguments by changing the cell membrane permeability. Saponins have also been suggested to be associated with the formation of saponin cholesterol insoluble complex (Francis *et al.*, 2002). Ibrahim and Srour Hany, 2013 reported that the presence of saponins was shown to reduce the level of cholesterol in nematode eggs and larvae. The reduction of cholesterol levels in nematodes affects the structural component of cell

membranes and indirectly might affect the motility of the nematodes. Coop & Kyriazakis, 1999 in their study suggested that tannins could bind to dietary protein and indirectly increase protein availability and enhance the immunological responses towards parasites.

The phytochemical analysis of the extract was not done in the present study. Amadi *et al.* (2018) evaluated the nutrient and phytochemical composition of jackfruit pulp, seed and leaves. The phytochemical composition showed that jackfruit pulp had phytic acid (6.14g/100g), oxalate(3.69g/100g), alkaloids(7.88g/100g), tannin(0.03g/100g) and flavonoid (3.91g/100g). Swamy and kalse, 2018 reported that the pulp portion of the fruit contains phytonutrients such as lignans, isoflavones, and saponins, and they have numerous health benefits. Uwanta E.J., *et al.* (2024), reported the presence of some phytochemicals such as alkaloids, saponins, phlobatannins, and Deoxy-sugar, in the fruit pulp.

Regarding tannins, flavonoids, and saponins, to our knowledge, there is currently no synthetic medication utilized as anthelmintics for the treatment of small ruminants. However, numerous studies on medicinal plants have indicated that these compound families may exhibit anthelmintic properties. As noted by Brunet *et al.* (2008), sainfoin extract, which is abundant in tannins, influences the unsheathing kinetics of *H. contortus* L3 larvae both *in vitro* and *in vivo*. Furthermore, the flavonoids and tannins found in the polar fraction of *Leuceana leucocephala* have been demonstrated to impact the migration of *H. contortus* L3 larvae (Ademola and Idowu, 2006; Hounzangbe-Adote *et al.*, 2005). Saponins derived from *Medicago polymorpha* and *Medicago sativa* have proven effective *in vitro* against gastrointestinal strongyles (Maestrini *et al.*, 2020).

Widiarso *et al.* (2021) in their study has observed that the crude aqueous extract of jackfruit leaves at various doses and observation times had a significant effect on *H. contortus* adult worms and altered the worm morphometry, especially the male and female body length,

cervical papillae width, spicula length in males and vulva flap length in females. A 1% dose of jackfruit leaf extract caused the greatest worm mortality.

As per the reports by the above mentioned authors, the phytochemicals present in the pulp portion of the fruit might be responsible for alteration in the integrity of the cuticle and death of the adult worms. The concentration of the ethanolic extract of the pulp portion of the Jack fruit that caused death of the adult worms could be further reduced in future studies.

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4. CONCLUSION

The ethanolic extract of fruit portion of the Jack fruit caused alteration in morphometry of adult *H. contortus* worms. At a very low concentration 10%, 100% mortality of worms was observed. Based on the *in vitro* adult worm mortality assay and morphometry examinations, the Jack fruit pulp ethanolic extract appears to be a potential anthelmintic agent against *H. contortus* infections.

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