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Full Length Article

## Anthelmintic activity of *Moringa oleifera* seed aqueous and ethanolic extracts against *Haemonchus contortus* eggs and third stage larvae

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## ABSTRACT

*Haemonchus contortus* is one of the prevalent and pathogenic ruminant parasites that has grown resistance to common anthelmintic treatment. This study evaluated the anthelmintic potential of *Moringa oleifera* seed ethanolic and aqueous extracts against *H. contortus* eggs and infective stage larvae (L3s). The efficacy of five extract concentrations (0.95, 1.95, 3.9, 7.8, and 15.6 mg/mL) were tested through egg hatch assay and larval motility test. Phytochemical tests were conducted to detect the different plant secondary metabolites in the extracts. In the ovicidal assay, the ethanolic and aqueous extracts showed 95.89% and 81.72% egg hatch inhibition at 15.6 mg/mL, respectively. The ovicidal activity of 15.6 mg/mL ethanolic extract was comparable with that of albendazole ( $p > 0.05$ ). The  $LC_{50}$  against the eggs was recorded at 2.91 and 3.83 mg/mL for ethanolic and aqueous extracts, respectively. In the larvicidal assay, the ethanolic and aqueous extracts exhibited 56.94% and 92.50% efficacy at 7.8 mg/mL, respectively. The larvicidal activity of 7.8 mg/mL aqueous extract was similar statistically with that of ivermectin ( $p > 0.05$ ). The  $LC_{50}$  against L3s was recorded at 6.96 and 4.12 mg/mL for ethanolic and aqueous extracts, respectively. The secondary metabolites detected were tannins in ethanolic extract and saponins in aqueous extract. Both extracts inhibited larvae formation inside the eggs and rendered the L3s immobile. Therefore, *M. oleifera* seed extracts contained plant bioactive compounds with anthelmintic property against the eggs and L3s of *H. contortus*.

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## 1. Introduction

Gastrointestinal nematodes (GINs) remain one of the most common ruminant production constraints in many developing countries. Among the GINs, *Haemonchus contortus* is the most pathogenic species in small ruminant [1]. This type of nematode sucks blood voraciously inside the goat's abomasum and causes severe anemia and anasarca. Hence, the parasite's pathogenic effect is attributed to severe blood loss that overwhelms the hematopoietic capacity of the animal thereby causing acute mortality [2]. Aside from its pathogenicity, *H. contortus* remains prevalent in countries with a tropical climate, such as the Philippines [3]. Therefore, haemonchosis suppressed the ultimate development of small ruminant subsector by contributing to production losses that are attributed to high neonatal mortality, high all-age morbidity, and high cost of treatment [4].

The treatment of haemonchosis has relied deeply on the repeated application of commercial and synthetic anthelmintics, for example, albendazole and ivermectin [5]. The term 'global worming' was thus coined describing the era of our dependency to and intensive use of such anthelmintics [6]. Regrettably, this act has resulted in the tremendous upsurge of anthelmintic resistant GINs, especially *H. contortus*. Resistant parasites have the resistant gene(s) of specific anthelmintic(s) which allow them to persist and cause havoc inside the animal's body. The worsening anthelmintic resistance has commonly been reported in goat farms and aggravated by the slow commercialization of novel anthelmintic compounds [6,7]. In the Philippines, benzimidazole resistance was considered widespread in many provinces of Luzon island [8].

The problem with commercial and synthetic anthelmintics is not confined to anthelmintic resistance alone. The indiscriminate application of such anthelmintics also threatens the public health by the increasing reports of drug residues in milk and meat. Moreover, the expensive product poses a question about its sustainability in developing countries where ruminant farming is common [4,9]. These drawbacks consequently give birth to new approaches in fighting GINs for sustainable livestock farming. One of these

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approaches is the exploration and screening of various plants for novel anthelmintic compounds [10].

Anthelmintics derived from indigenous plants have pragmatic features for small hold farmers who cannot afford the commercial product, and for large commercial farmers who are shifting to organic farming [11,12]. Furthermore, herbal anthelmintics as a natural product are implicated to least likely bioaccumulate in the tissues of animal and the environment [13]. They are likewise considered eco-friendly and biodegradable [13,14]. Above all, multiple bioactive compounds present in herbal anthelmintics may translate to multiple mechanisms in killing the parasites, which then limit the likelihood of developing anthelmintic resistance [15].

*Moringa oleifera* is one of the important plants in the Philippines utilized for food and traditional medicine. Previous studies revealed the array of its bioactive compounds with nutritive and medicinal values present in the leaves, seeds, roots, barks, and flowers [16]. In addition, this plant was utilized in the treatment of malaria, leishmaniasis, trypanosomiasis, schistosomiasis, dracunculiasis, and filariasis thus suggesting its inherent antiparasitic property [16,17]. This property was further supported by few studies utilizing the leaf and seed extracts against the gastrointestinal parasites of the ruminant. Tayo et al. [18] concluded that the infused and macerated aqueous and ethanolic leaf extracts exhibited varying level of ovicidal and larvicidal activities against the eggs (fresh and embryonated) and larvae (L1 and L2) of *H. contortus*. A field study further demonstrated that supplementation of leaves may suppress the burden of strongyle worms in naturally infected cross-bred Xhosa lop eared goats [19] and West African Dwarf goats [20]. Aside from the leaves, the seed extract of *M. oleifera* was also evaluated against *H. contortus* [20]. Salles et al. [21] showed that the seed fraction of low molecular weight (<12 kDa) contained potential molecules responsible for over 90% egg hatch inhibition.

To the best of our knowledge, the study of *M. oleifera* seeds against *H. contortus* is very limited and has no data pertaining to its larvicidal activity. The current study utilized a different plant extraction approach and evaluated the ethanolic and aqueous extract of *M. oleifera* seeds against the eggs and L3s of *H. contortus*. We further tested the extracts for secondary metabolites that might be responsible for its anthelmintic activity.

## 2. Materials and methods

### 2.1. Collection and extraction of *M. oleifera* seeds

Young pods containing the seeds of *M. oleifera* were collected from Brgy. Sto. Rosario, Baybay City, Leyte. The freshly collected seeds were air-dried for three days to remove 60% of its moisture content. The dried seeds were pulverized and extracted following the protocol of Fernandez et al. [22]. In brief, the powdered seeds were soaked in 99.5% ethanol at the ratio of 1:3 (w/v) and allowed to stand protected from light at 27 °C for 48 h. The ethanol extract was sieved and filtered using a muslin cloth and Whatman™ Grade 54 filter paper, respectively. The ethanol solvent was evaporated at 40 °C in a vacuum rotary evaporator until the remaining volume of the extract was 3% of the original. The product of the ethanol extraction was called *M. oleifera* seed ethanolic extract (MSEE). A 1% dimethyl sulfoxide (DMSO) was added to enhance the emulsification of MSEE. Following the previous procedure, the seed residue was re-extracted using distilled water as a solvent and concentrated the extract at 60 °C in a vacuum rotary evaporator to obtain the *M. oleifera* seed aqueous extract (MSAE).

### 2.2. Phytochemical screening

The ethanolic and aqueous extracts were tested for the presence of alkaloids (Dragendorff's reagent), flavonoids (Bate-Smith and Metcalf test), saponins (froth test), terpenoids (Salkowski test), and tannins (ferric chloride reaction and gelatin test) [4,22].

### 2.3. Egg hatch test

All adult *H. contortus* were recovered from the abomasum of goat slaughtered in Baybay City Abattoir. For the preparation of egg suspension, all female *H. contortus* were morphologically selected and macerated to liberate the eggs. The eggs were successively sieved and finally diluted with phosphate-buffered saline (PBS). Every treatment was triplicated in which a single replicate contained 10–15 random sample of eggs in 10 µL PBS. These eggs were exposed to *M. oleifera* seed extracts (0.95, 1.9, 3.9, 7.8, and 15.6 mg/mL) and control groups (0.5% DMSO, 0.5% ethanol, PBS, and 0.5% albendazole). These were humidly incubated in a room at 27 °C for 72 h. We stopped the development of eggs by adding a drop of Lugol's iodine and counted the number of eggs that remained morulated, showed 'larva failing eclosion', and turned into free L1 [23] (Fig 1). The ovicidal activity was expressed based on the percentage of eggs that failed to develop and hatch [23].

### 2.4. Larval motility test

The procedure for the culture of L3s was based on the technique described by Rupa and Portugaliza [3], while the *in vitro* larvicidal assay was adopted from Fernandez et al. [22]. There were six replications per treatment and for each replicate an amount of 10 µL PBS with 10–15 alive L3s were randomly pipetted. The L3s were exposed to *M. oleifera* seed extracts (0.95, 1.9, 3.9, 7.8, and 15.6 mg/mL) and control groups (0.5% DMSO, 0.5% ethanol, PBS, and 0.5% ivermectin). These were humidly incubated at 27 °C for 3 h. After 3 h, we recorded the motility of L3s by gentle prodding. Afterward, we added a drop of 1% Delafield's Hematoxylin stain and re-incubated the set-up for 24 h. The L3s were re-examined under the microscope for the uptake of stain. We categorized immobile larvae based on motility upon prodding and identified the larvae with cuticle damage based on the uptake of the red stain (Fig 2). The immobile larvae were either dead or weakened; hence, the larvicidal activity was expressed on the percentage of immobile L3s after exposure [22].

### 2.5. Statistical analysis

We calculated the lethal concentrations (LC<sub>50</sub>) and 95% fiducial limits (95% FL) of *M. oleifera* seed extracts using the probit analysis and analyzed the difference between treatment groups using one-way ANOVA and Tukey's HSD in IBM SPSS version 21. All results were considered statistically significant when  $p < 0.05$ .

## 3. Results

Table 1 shows the *in vitro* anthelmintic activity of *M. oleifera* seed ethanolic (MSEE) and aqueous (MSAE) extracts against the eggs and L3s of *H. contortus*. Except for the larvicidal activity of MSAE, the rest of the *M. oleifera* seed extracts displayed its anthelmintic activity in a dose-dependent manner (Fig. 3). Furthermore, the secondary metabolites detected in the ethanolic and aqueous extract were only tannins and saponins, respectively. Alkaloids, flavonoids and terpenoids were undetected from MSEE and MSAE.

In the ovicidal assay, the ethanolic extract exhibited the highest or 95.89% efficacy at 15.6 mg/mL. This result was statistically com-

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