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Evaluation of enhanced virgin coconut oil and senduduk (*Melastoma malabathricum*) as anthelmintics against caprine strongyle nematodes

Basripuzi, H.B.[1*], Sani, R.A.[2], Ariff, O.M.[3] and Chandrawathani, P.[4]
[1]Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Locked Bag 36, 16100 Pengkalan Chepa, Kota Bharu, Kelantan, Malaysia
[2]Research Centre for Ruminant Diseases, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[3]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[4]Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia
[*]Corresponding author email: basripuzi@yahoo.com; basripuzi@umk.edu.my
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Abstract. A study was conducted to evaluate the anthelmintic properties of enhanced virgin coconut oil (EVCO) and senduduk (*Melastoma malabathricum*) plant against strongyle nematodes in goats. Two preparations of 10% EVCO dissolved in 90% virgin coconut oil and 10% EVCO dissolved in 90% palm oil, were given orally to two groups of mixed breeds goats. The efficacy test indicated that EVCO was insufficiently active as an anthelmintic. Four concentrations of senduduk solution (1.25, 2.5, 5.0 and 10 mg ml^{-1}) were compared with a control and albendazole in an *in vitro* test for larvicidal effect. There was no significant larval mortality using senduduk solution. An *in vivo* test of senduduk was conducted by comparing three groups of goats, namely control, levamisole and treatment groups that were given a daily oral dose of senduduk crude extract with 1g kg^{-1} from Day 0 to Day 12 and 2 g kg^{-1} from Day 13 to Day 30. This efficacy test with senduduk also gave negative results. The findings obtained indicated that EVCO and senduduk were ineffective as anthelmintics against caprinestrongyle nematodes at the concentrations used.

INTRODUCTION

Gastrointestinal nematode infection is one of the major problems facing the small ruminant industry in Southeast Asia and is responsible for heavy morbidity and mortality mainly caused by *Haemonchus contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. (Sani & Gray, 2004). Nearly a decade ago, the annual economic impact of small ruminant parasitism in selected Asian countries including Thailand, Malaysia, Nepal and Philippines was estimated in the region of $US20 million (McLeod, 2004). Chemical anthelmintics remain the key approach to control nematode populations and their indiscriminate use has ultimately contributed to the development of anthelmintic resistance (Chandrawathani, 2004). Anthelmintic resistance is generally considered of greater importance in the warmer regions of the world (Waller, 2002). In Malaysia, anthelmintic resistance has been reported since the early 1990s with the first report by Dorny *et al.* (1994). This spurs the need to reduce the reliance on chemical anthelmintics and intensify investigations for alternative control approaches.

Plant-based dewormers have been suggested by Jabbar *et al.* (2006a) as one of the alternative strategies to control and/or delay the onset of anthelmintic resistance. The other strategies include resistant animal breed, grazing management and anthelmintic treatments, nutrition and parasite interactions, antiparasitic vaccines

and biological control. A wide variety of plant-based dewormers have been reported for their use in livestock industry (Akhtar *et al.*, 2000; Diehl *et al.*, 2004; Jabbar *et al.*, 2006b; Hussain *et al.*, 2008; Farook e*t al.*, 2008). In Pakistan extensive works had been done on anthelmintic activity of plant-based dewormers in sheep by Iqbal *et al.* (2004, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2010).

There is a resurgent interest in the screening and application of plant remedies as alternatives to traditional commercial anthelmintics (Schillhorn van Veen, 1997) which is supported by Waller (2006). In reality, plant remedies have been used in livestock husbandry since ancient times in many countries to treat gastrointestinal parasitism (Githiori *et al.*, 2006), particularly for resource-poor farmers as an alternative to chemical control (Waller & Thamsborg, 2004). The nematocidal activity of tanniferous plants and their condensed tannins has gained attention as a potential non-chemical control strategy (Ketzis *et al.*, 2006). In Malaysia, neem (*Azadirachta indica)* and cassava (*Manihot esculenta*) leaves have been shown to reduce worm burdens in sheep (Chandrawathani *et al.*, 2002, 2006; Nurulaini *et al.*, 2009).

Health benefits and disease prevention attributed to coconut (*Cocos nucifera*) products in various forms have been described by DebMandal & Mandal (2011). Anthelmintic activity of coconut product was observed *in vitro* on acetate extract obtained from the liquid of green coconut fiber (Oliveira *et al.*, 2009). Sato *et al.* (2004) found that medium-chain triglyceride (MCT) had an anticoccidial effect in calves. Further study revealed similar anticoccidial efficacy without side effects observed on ruminal protozoa via abomasal MCT feeding (Sato *et al.*, 2009). Thus the anthelmintic effect of enhanced virgin coconut oil (EVCO), which contains MCT was investigated.

In vitro anthelmintic activity of senduduk extracts had been observed on inhibition of eggs hatching and larval development of *Haemonchus contortus* in Indonesia (Suteky & Dwatmadji, 2011). In Malaysia and according to folklore, the whole plant of senduduk (*Melastoma malabathricum)* is used to treat diseases and ailments (Joffry *et al.*, 2012). Senduduk is commonly consumed by Jah Hut people of Malaysia to treat diarrhoea and its leaves have been proven to have anti-diarrhoeal effects in mice (Sunilson *et al.*, 2009). The goat farmers in Kelantan, Malaysia also claim that senduduk has anthelmintic effects as it cures diarrhoea in goats. A questionnaire survey conducted prior to the study revealed four out of five farms give senduduk as a dewormer when goats showed diarrhoea.

Therefore the objectives of this study were to determine the anthelmintic effect of EVCO by *in vivo* testing and to evaluate *in vitro* and *in vivo* anthelmintic effects of *M. malabathricum* on caprinestrongyle nematodes.

MATERIALS AND METHODS

Study sites, animals and parasite infection

EVCO testing was conducted from May to June 2011 at two locations; on a goat farm at Kuala Lipis, Pahang, Malaysia and at the Goat Unit, Livestock Section, University Agricultural Park, Universiti Putra Malaysia (UPM). Twenty-four goats of both sexes ranging in age from less than 1 year to more than 5 years were selected from a goat herd that had been raised semi-intensively in elevated wooden slatted floor houses at the Kuala Lipis farm. The goats were of Katjang, Jamnapari, Boer and Saanen breeds. They were fed commercial pelleted feed in the morning and left to graze around the shed in the afternoon. The goats were provided water *ad libitum* and occasionally fed freshly cut Napier grass (*Pennisetum purpureum*). They were not dewormed in the 12 months before the commencement of the study. Albendazole was previously used on the farm. Another 12 goats of mixed-sex and the same age range as the Kuala Lipis goats reared in a comparable environment and under similar management at the UPM farm were also selected. These goats consisted of Katjang, Boer and Saanen breeds. They were fed freshly cut Napier grass, a commercial

pelleted feed and provided water *ad libitum*. This farm was selected due to the high faecal egg count (FEC) in the goats although the goats were dewormed 1 month previously.

Eleven goats from the Kuala Lipis farm and eight goats at UPM were identified to have gastrointestinal nematode infections by the modified McMaster technique (Lyndal-Murphy, 1993) before the start of the study. The FEC of these goats ranged from 200 to 4200 eggs per gram (epg) of faeces with a mean FEC of 939 epg. The goats from both locations were divided into two groups for EVCO testing. Five goats from Kuala Lipis and four goats from UPM were assigned into Group 1. Six other goats from Kuala Lipis and the remaining four goats from UPM were allocated into Group 2.

The study on senduduk was also carried out at the Goat Unit, UPM from December 2011 to January 2012. Eighteen goats of both sexes ranging from less than 1 year to 2-years old were confirmed to be infected with gastrointestinal strongyle nematodes prior to the *invivo* study. The FEC of these goats ranged from 300 to 4800 epg with a mean of 1461 epg. The goats were divided equally into three groups. All goats included in this study had FECs more than 150 epg based on guidelines by Coles *et al.* (1992).

Plant materials and solution preparation

EVCO and virgin coconut oil (VCO) were obtained from Malaysian Agriculture and Research Development Institute (MARDI) at Serdang, Selangor. A solution made up of 10% EVCO dissolved in 90% VCO was prepared by adding 35 ml EVCO to 315 ml VCO. Nevertheless cooking oil of palm oil origin would be more affordable by most small holder farmers compared to the cost of VCO and was therefore included in the study. Another solution containing 10% EVCO dissolved in 90% palm oil was prepared by adding these oils with similar volumes as EVCO and VCO solution.

Fresh plant materials of senduduk consisting of stems, leaves, flowers and fruits were collected from its natural habitat in Benta, Kuala Lipis, Pahang (3° 58' 39.81"N, 101° 59' 15.91"E). Voucher specimen SBID 016/11 was deposited in the herbarium at the Forest Research Institute Malaysia (FRIM), Kepong, Selangor and identified as *M. malabathricum* L. from the family Melastomataceae. The plant material was air dried under shade for 4–5 days. The leaves, flowers and fruits were separated from the stems, ground into fine powder and kept in an air-tight container. The dried powder sample weighing 5.0 g was also screened for phytochemical constituents at FRIM. The sample labelled as JN 40/11 was analysed for the presence of saponins, flavonoids, condensed tannins, triterpenes and steroids.

In the *in vitro* study, 50 mg senduduk powder was added to 1 ml Tween 80 and mixed using a magnetic stirrer for 24 hours. The stock solution was serially diluted in distilled water to produce working solutions of 1.25, 2.5, 5.0 and 10 mg ml^{-1}. The positive control was prepared by adding 50 µl albendazole in 950 µl distilled water to produce a 5 µg ml^{-1}albendazole solution. The negative control was prepared by mixing 4 ml distilled water and 1ml Tween 80.

In the *in vivo* study, 1g kg^{-1} animal body weight of senduduk extract was used. Therefore for six goats in the treated group weighing a total of 169 kg, 169 g senduduk powder was completely immersed in boiling water (about 400ml) in a covered container till the colour of the solution did not deepen further (for 30 minutes). The extract was obtained by filtering the infusion through a cloth strainer. The extraction process was done fresh daily in the morning at the Parasitology Laboratory, Faculty of Veterinary Medicine, UPM from Day 0. As no reduction in FEC was observed by Day 12, the extract concentration was increased to 2 g kg^{-1} on Day 13 until Day 30.

***In vitro* technique**

Infective third-stage larval (L3) stocks of 20 µl containing approximately 18 *H. contortus*were pipetted into each well of a 96-well microtitre plate. Positive and negative control solutions of 20 µl were added to 16 wells each. Senduduk working solution was also prepared as 16 replicates for each concentration. The well plate was covered with a tape, incubated at 20ºC for 24 hours and observed under an inverted

microscope for 24 hours post-incubation. Death of L3 was characterised by lack of motility and a straight body while surviving L3 were confirmed by motility. The number of dead and living L3 in each well were counted and recorded. Larval mortality percentages were calculated by dividing the total number of dead L3 to the total number of L3 per group.

***In vivo* technique**

Pre-treatment faecal samples were collected per rectum from each goat on Day 0 prior to oral administration of EVCO treatments. Group 1 were given 10% EVCO dissolved in 90% VCO while Group 2 were given 10% EVCO dissolved in 90% palm oil. Volume for each treatment was 1 ml 5.0 kg^{-1} of bodyweight. Post-treatment faecal samples were collected on Day 10. The efficacy of EVCO treatment was measured by the faecal egg count reduction test (FECRT) using arithmetic means based on a calculation formula by Abbott (1925) as reported byPandey&Sivaraj (1994):

FECR% = (Pre-treatment epg – Post-treatment epg) / Pre-treatment epg × 100

In the senduduk study, Group 1 as the negative control received no treatment. Group 2 as the positive control received a single oral dose of levamisole 0.675 mg kg^{-1} of bodyweight on Day 0 pre-treatment and Group 3 was given a daily oral dose of 1g kg^{-1} of crude extract of senduduk from Day 0 to Day 12 and 2 g kg^{-1} from Day 13 to Day 30. The goats were treated prior to be fed by cut and carry system in the morning. Faecal samples of each goat in Group 1 and Group 3 were collected per rectum in the morning from Day 0 to Day 30 after treatment. Collection of faecal samples from Group 2 (levamisole) was discontinued when no eggs were detected from each goat for 3 days. The final (post-treatment) faecal samples were collected from goats in Group 1 and Group 3 on Day 31. Faecal samples were kept in airtight containers, brought to the laboratory and evaluated by the modified McMaster technique (Lyndal-Murphy, 1993) on the same day of collection to determine FEC. Samples were also pooled and subjected to faecal culture for 7 days at 22 to 27ºC as described by Coles *et al.* (2006). Faecal cultures of Groups 1 and 3 were prepared on Days 0, 11, 21 and 31 while only a pre-treatment faecal culture was conducted for Group 2. The L3 were collected for identification (to the genus level) and enumeration.

Pre- and post-treatment blood samples were collected for biochemistry analysis on urea and gamma-glutamyltransferase(GGT) to evaluate any effect on kidney and liver function due to the administration of senduduk.

Statistical analysis

The efficacy tests on EVCO and senduduk followed the FECRT method of Coles *et al.* (1992). Statistical analyses for testing treatment group differences were performed using IBM SPSS Statistics ver. 19. Comparisons between groups for mean percentages of larval mortality in *in vitro* were performed by one-way ANOVA followed with the Tukey honestly significance difference test for significant group differences.

RESULTS

The FECRT results for EVCO treatments are shown in Table 1. There was a 55% reduction in FEC in goats given 10% EVCO dissolved in 90% VCO in the Kuala Lipis farm. However in another treated group at Kuala Lipis and both groups at UPM, higher FECs after treatment resulted in negative reduction percentages ranging from -40% to -145%.

Mean larval mortalities of *H. contortus* in control, albendazole and different concentrations of senduduk in the *in vitro* study are shown in Table 2. The treatment effects on larval mortality differed significantly across the six groups ($p = 0.02$). However, statistical analysis showed only the positive control group (54.75 ± 3.53) gave significantly higher ($p < 0.05$) larval mortality than the negative control group (33.94 ± 3.49).

Table 1. Means and standard errors for FEC before and after EVCO treatments with FECRT results at two farms

Farm	Treatment	N (goats)	Mean FEC (epg)		FECRT (%)
			Pre-treatment	Post-treatment	
Kuala Lipis	10% EVCO + 90% VCO	5	400 ± 190	180 ± 80	55
	10% EVCO + 90% palm oil	6	517 ± 87	1267 ± 380	-145
UPM	10% EVCO + 90% VCO	4	750 ± 202	1675 ± 825	-123
	10% EVCO + 90% palm oil	4	1425 ± 437	2000 ± 618	-40

Table 2. Mean larval mortality percentages of *Haemonchus contortus* in albendazole and senduduk treatment groups from *in vitro* study

Larval mortality	Treatment					
	Control	Albendazole	Senduduk concentration (mg ml^{-1})			
			1.25	2.5	5.0	10.0
Mean±se	33.94^{a}±3.49	54.75^{b}±3.53	40.63±5.19	38.06±3.78	38.31±4.14	40.25±5.01

^{a,b}Means in the same row with different superscripts differ significantly at $p < 0.05$

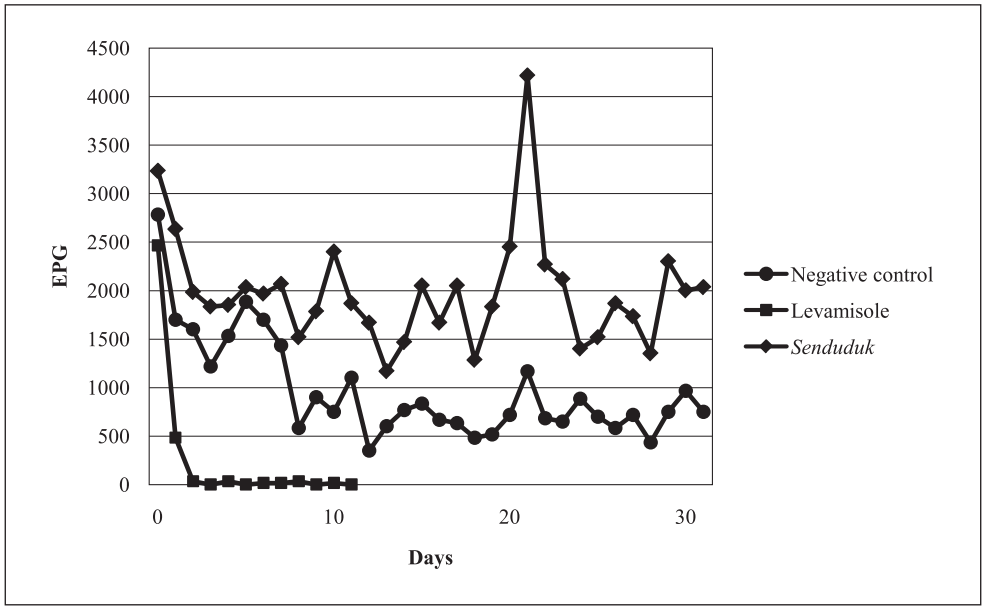

Figure 1. Mean FEC for control, levamisole and senduduk groups in *in vivo* study

Comparisons between groups given senduduk and the other two groups were not statistically significant ($p > 0.05$).

Patterns of mean FEC for control, levamisole and senduduk treated groups in the *in vivo* study are shown in Figure 1. All groups showed reduction in post-treatment mean FEC compared to Day 0 pre-treatment mean FEC. The group given levamisole had 0 epg by Day 11. Thus faecal sampling for

Table 3. FECRT results of levamisole and senduduk treated groups in *in vivo* study

Treatment	Days post-treatment		
	11	21	31
Levamisole	100	NT[1]	NT
Senduduk	-220	-261	-171

[1]NT = Not taken

this group was discontinued. There were almost similar trends in mean FEC of the negative control group (350 ± 118 to 2783 ± 1179) and senduduk group but the mean FEC of the senduduk groups remained the highest (1167± 295 to 4217 ± 1093) throughout the study.

Table 3 shows FECRT results of levamisole and senduduk treated groups in the *in vivo* study. Days 11, 21 and 31 were used to observe FECRT at 10-day intervals for the senduduk groups and 100% efficiency of levamisole treatment on Day 11. There was no reduction in mean FEC of the senduduk treatment groups as indicated by their negative results. Biochemistry analysis revealed no adverse effect on kidney and liver function due to the administration of senduduk.

Table 4 shows percentages of infective larval population by nematode genera yielded from faecal culture of control, levamisole and senduduk treatment groups on Days 0, 11, 21 and 31. *Haemonchus contortus* was the predominant species in all groups followed by *Trichostrongylus* sp. and *Oesophagostomum* sp.

DISCUSSION

There is no specific guideline to evaluate the efficacy of plant anthelmintic activity to date. Therefore in the present study, assessment of the plant anthelmintic efficacy was based on the existing guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) as recommended by Githiori *et al.* (2006) and Hoste *et al.* (2008). In the WAAVP guidelines by Wood *et al.* (1995), efficacy is defined as highly effective if FECRT is over 98%; effective if 90–98%; moderately effective if 80–89% and insufficiently active when less than 80%.

In the EVCO study, 10% EVCO was used for both treatments as recommended by MARDI as the manufacturer, who conducted their studies on animals by dissolving it in

Table 4. Percentage composition of nematode genera from faecal larval cultures in levamisole and senduduk treatment groups from *in vivo* study

Day	Infective larvae (L3) by nematode genera (%)	Control	Levamisole	Senduduk
0	*Haemonchus contortus*	69	77	91
	Trichostrongylus sp.	31	23	9
	Oesophagostomum sp.	–	–	–
11	*Haemonchus contortus*	81	NT[1]	96
	Trichostrongylus sp.	19	NT	4
	Oesophagostomum sp.	-	NT	-
21	*Haemonchus contortus*	83	NT	94
	Trichostrongylus sp.	13	NT	6
	Oesophagostomum sp.	4	NT	–
31	*Haemonchus contortus*	98	NT	94
	Trichostrongylus sp.	2	NT	6
	Oesophagostomum sp.	–	NT	–

[1]NT = not taken

90% VCO. In this study, the negative reduction percentages of FECRT shown in the four groups from two different farms indicated that EVCO was ineffective as an anthelmintic at the concentration used. The reduction in FEC as shown in one group given 10% EVCO dissolved in 90% VCO at the Kuala Lipis farm based on the definition by Wood *et al.* (1995) showed that EVCO was insufficiently active as an anthelmintic. MCT showed anti-coccidial effect in calves at daily oral dosages between 40-100 ml until disappearance of coccidialoocyts (Sato *et al.* 2004). Thus a single oral dose of EVCO which also contained MCT at a concentration of 1 ml 5.0 kg^{-1} of bodyweight may be insufficient to exert anthelmintic effects in goats. *In vivo* test of ethyl acetate extract obtained from the liquid of green coconut husk fiber also showed no activity against gastrointestinal nematodes in sheep (Oliveira *et al.*, 2009). Nonetheless this study evaluated worm burden by total worm count whereas the present study evaluated worm count by FECRT.

In the senduduk *in vitro* study, it was expected that albendazole as the positive control would have the highest larval mortality percentage while the negative control would have the lowest larval mortality percentage. However the low mortality in the albendazole group provided evidence of suspected resistance in the larval strain used. According to Jackson & Hoste (2010) the albendazole concentration used in this study is sufficient to prevent hatching of nematode eggs, which may also affect the larvae survival. This study suggested that senduduk was not effective as a larvicide at the concentrations tested.

Investigation by *in vivo* testing was still considered justifiable despite the insignificant findings on larval mortality percentages of the senduduk groups that were slightly higher than the negative control group. According to Hoste *et al.* (2008), the main disadvantage of *in vitro* assay is the difficulty in the interpretation of the results due to major differences between *in vitro* and *in vivo* conditions with considerable physiological changes and possible degradation or transformation of active compounds in the animals. Moreover, virtually any substance including common salt added to an *in vitro* study would have a damaging effect on larvae (Peter Waller, personal communication).

Senduduk was ineffective as an anthelmintic as indicated by its negative FECRT results and its mean FEC pattern throughout the study. Additionally senduduk was unable to eliminate *H. contortus* that constantly remained as the most dominant nematode. According to Sani & Gray (2004), plants that were successfully used in eliminating endoparasites may well be acting as laxatives and not strictly by an anthelmintic effect. In the present study, levamisole was still highly effective as an anthelmintic. Githiori *et al.* (2006) suggested that a criterion be established to adequately evaluate plant anthelmintic activity because the majority of anthelmintic effects of plant remedies are lower than that reported for chemical anthelmintics.

In a previous study by Suteky & Dwatmadji (2011), crude aqueous extract of senduduk caused immotility about 66.25% within 2 hours and completely inhibited motility of adult *H. contortus* at 8 hours after exposure. The extract was prepared by boiling the senduduk powder for 1.5 hours in 500 ml water. Higher concentration of the extract inhibited adult motility much earlier. In the present study, *in vitro* test was done by using senduduk stock solution on L3 *H. contortus*. Although lower concentrations of senduduk stock solution were used in the present study than senduduk crude aqueous extract used by Suteky & Dwatmadji (2011), larval mortality was observed between 38 to 40% at 24 hours after exposure. No difference was observed in larval mortality percentages between different concentrations of the stock solutions. The differences occurred in both studies may be due to the use of different larval stages that were treated by different senduduk treatments (crude aqueous extract and stock solution). Nevertheless both *in vitro* studies showed potential anthelmintic properties of senduduk but in contrary to the *in vivo* test in the present study.

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