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EFFICACY OF NEEM, SABAH SNAKE GRASS LEAVES WATER EXTRACTS AND NEEM DECOCTION AGAINST GASTROINTESTINAL PARASITES IN LOCAL GOATS

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ABSTRACT. This is a study conducted to test the effectiveness of *Azadirachta indica* (Neem), *Clinacanthus nutans* (Sabah Snake Grass) water extracts and neem decoction against natural gastrointestinal helminths in 4 groups of goats for a period of 4 weeks. This study was done on 24 experimental goats which were randomly chosen from a private farm at Gopeng, Perak. They were divided into four groups; control (n=6) and three treatment groups (n=6). Faecal egg counts and faecal culture to identify the L_3 larvae were done and recorded weekly from January until February 2015. Blood parameters such as FAMACHA and PCV were observed and recorded twice at the beginning and end of the study. The FEC results indicated that none of the three treatments were significantly different to control group ($P > 0.05$).

INTRODUCTION

The livestock industry is a significant component of the Malaysian agricultural sector. According to the Department of Veterinary Services, Malaysia (2012), the need for mutton, eggs and milk are expected to register more than 30% increase in 2020. In order to meet the increasing demand, we are importing goat and sheep meat from Australia, New Zealand and other countries. However, research have shown that helminth infection by gastrointestinal nematodes such as *Haemonchus contortus*, *Oesophagostomum* spp., *Cooperia* spp. and *Trichostrongylus* spp. cause serious losses to the livestock sector (Waller, 2006). Chemical drugs (anthelmintic) have been used for several decades to combat these gastrointestinal nematodes. Continuous usage of anthelmintic drugs leads to serious cases of anthelmintic resistance. Hence, an alternative or non-chemical approach to control the gastrointestinal nematodes in small ruminants such as ethnoveterinary therapy is necessary.

Ethnoveterinary medicine is a systematic approach that covers the folk's knowledge, beliefs, skills,

practices, methods, technology, resources and social organisation related to the healthcare of animals handled by human beings (McCorkle, 1986). In this study, two medicinal plants were used which are *Azadirachta indica* (Neem) and *Clinacanthus nutans* (Sabah Snake Grass) to control the population of nematodes in small ruminants. Neem tree (*Azadirachta indica*) which is also known as the wonder tree of India is a native to India and Pakistan and every part of this tree is noted to have medicinal properties. The neem leaves are characteristically medium green in colour, unpaired pinnate, asymmetric and are serrate leaflets. The discovery of Azadirachtin with its outstanding biological activity initiated the chemical exploration of every part of neem trees and this active compound has hindrance, anti-ovipositat, antifeedant, growth-disrupting, fecundity and fitness reducing properties on insects (Schmutterer, 1990). Previous study showed that neem products particularly neem juice, neem decoction and neem capsule have the ability to reduce gastrointestinal worm egg counts from 40-60% during the study period (Chandrawathani *et al*., 2013). Sabah Snake grass which is scientifically known as *Clinacanthus nutans* belongs to the family Acanthaceae and is a famous medicinal plants in Thai folklore medicine (Yong *et al*., 2013). It is also known as *You Dun Chao* in Mandarin or *Belalai Gajah* in Malay and grows well in tropical countries such as Malaysia and Thailand. According to Health Care Asia Daily (2013), Sabah Snake grass was named as such for its initial use in treating snake bites as it was believed to neutralize the poison from snake. However, in recent years it is widely known for its healing properties such as cure for cancer and kidney failure. This fruitless plant has leaves which looks like shoots of wild grass and its stem appear plastic-like and the growth can be multiplied by stem-cutting. Fresh matured leaves of *C. nutans* has been used for quite some time by traditional doctors in Thailand to treat skin rashes, insect bite as as herpes simplex virus (HSV) and varicella-zoster virus (VZV) lesions (Sakdarat et al., 2006). The aim of this study conducted was to determine the efficacy of the herbal extracts prepared against the gastrointestinal nematodes in local goats. Ethnoveterinary approach will be the best option to curb helminthosis and help farmers to manage their livestock at low cost.

METHODS AND MATERIALS

Study area and animals

This study was conducted upon local goats at a private farm, the Yayasan Darul Hikmah Farm located in Gopeng, Perak from January 2015 to February 2015. This study involved 24 adult local goats of Jamnapari cross and Boer cross that were reared semi-intensively. The experimental goats were grouped randomly into four groups; one for control and three for treatment with 6 goats in each and were tagged at the ears for differentiation

purpose. The control were not given any treatment, while the other three groups were treated with *A. indica* extract, *A. indica* decoction and *C. nutans* extract. Faecal egg counts were taken once a week for a period of 4 weeks.

Preparation of Leaves Extract and Decoction

Neem extract (Azadirachta indica)

The method used to prepare Neem extract was as suggested by Chandrawathani et al. (2013). The neem trees were commonly available and the leaves were collected from the trees on the grounds of Universiti Sains Malaysia, Penang, Malaysia. The neem leaves were freshly harvested, washed and carefully cleaned using running tap water and then left to air-dry under the shade for two days (48 hours). Then the dried leaves were grinded into powder using an electric grinding mill. The resulting neem leaves powder was stored in air-tight containers until usage at room temperature.

The neem leaves powder (180 g) was mixed with distilled water (900 ml) at a ratio of 1:5 in 1500 ml conical flask. A total amount of 1320 g of powdered Neem leaves was mixed with 6660ml of distilled water. By using water bath (Stuart, waterbath-RE300B), the mixture was heated at a temperature of 70 °C for 6 hours and the resulting neem leaves extract was filtered using filter paper (Whatman No. 1, USA). The extract was concentrated using a rotary vacuum evaporator (Heidolph Rotary Evaporator) at 40 °C and left to dry at 40 °C in the oven for about 48 hours (2 days). The resulting sediments were scraped and stored in the sterilised universal bottle at 4 °C until used.

A total of 30 g of Neem leaves paste extract was diluted with 300ml of distilled water producing a liquid extract. 50 ml of the water extract equivalent to 5000 mg of leaves extract were given twice to each goat with an average body weight of 40 kg in week 1 and week 2. Hence, the dose rate is 125 mg of Neem extract/kg body weight. The liquid extract was given orally to each goat using a 50 ml syringe.

Neem leaves decoction (Azadirachta indica)

Neem decoction was prepared based on the method recommended by Chandrawathani et al. (2013). Fresh matured neem leaves were plucked from the Neem trees in the grounds of the Veterinary Research Institute, Ipoh, Malaysia. A total of 24 g of Neem leaves were weighed and soaked in 240 ml of distilled water for 48 hours (2 days) in a 1000 ml beaker. The decoction was stored in a clean bottle after filtration at room temperature until used. A volume of 40 ml of Neem decoction equivalent to 4000 mg of Neem extract were given twice to each goat with an average weight of 40 kg in week 1 and week 2. Thus the dose rate was 100 mg/kg of Neem decoction per kilogram body weight. This was also given orally using 50 ml syringe.

Sabah Snake Grass extract (Clinacanthus nutans)

The Sabah Snake Grass extract was bought from You Dun Chao Healthcare Products Sdn Bhd 838860-W. The extract contained 70 percent of maltose and 30 percent of Sabah Snake Grass (Plate 3.10). The extract was prepared as described by Yong *et al.* (2013). A volume of 50 ml of liquid extract is equivalent to 5000 mg of leaves extract were given to each goat with an average weight of 40 kg. The extracts were given orally using a 50 ml syringe twice to each goat in week 1 and week 2.

Specimen Collection and Laboratory Techniques

Fresh rectal faecal samples were collected from each goat every week and the McMaster technique modified from the Manual of Veterinary Parasitological Laboratory Technique, (1986) was used for faecal egg counts. The faecal culture was also done to identify the third stage larvae of helminths. The Faffa Malan Chart (FAMACHA) technique and percentage of packed cell volume (PCV) were determined during the pre- and post-treatment to evaluate anaemia cases among the experimental goats.

Statistical analysis

Microsoft Excel 2007 and IBM SPSS Statistic 20 were used to generate descriptive statistic and graphs for the purpose of data analysis. The variation in FEC were determined using Anova One-Way with Post Hoc Multiple Comparison (Tukey test). The significant level for statistical tests were set to $p < 0.05$.

RESULTS

Faecal egg counts (FEC) were recorded weekly for a period of 4 weeks at the Yayasan Darul Hikmah Farm with treatments given at the first and second week. Generally, most of the treatments including the control group showed a significant reduction in the average egg per gram (epg) at the second week except for the group treated with Neem leaves decoction. Figure 1 show that at the

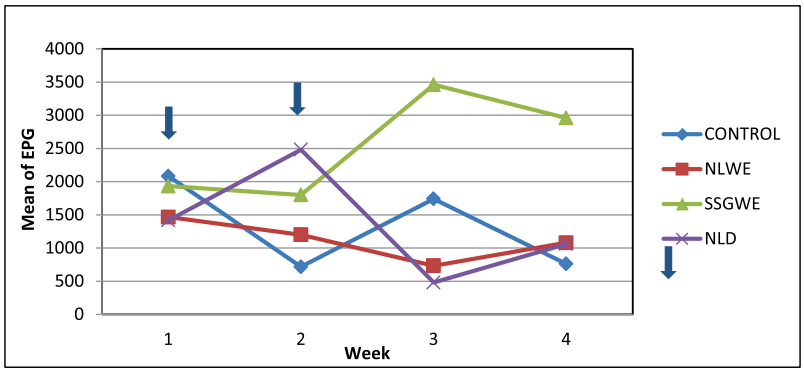
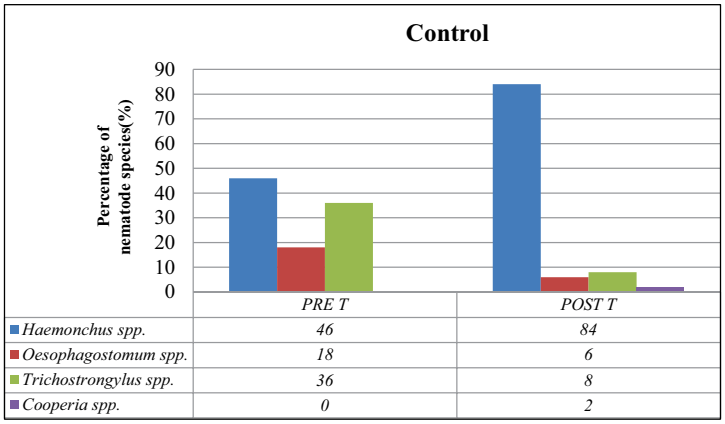
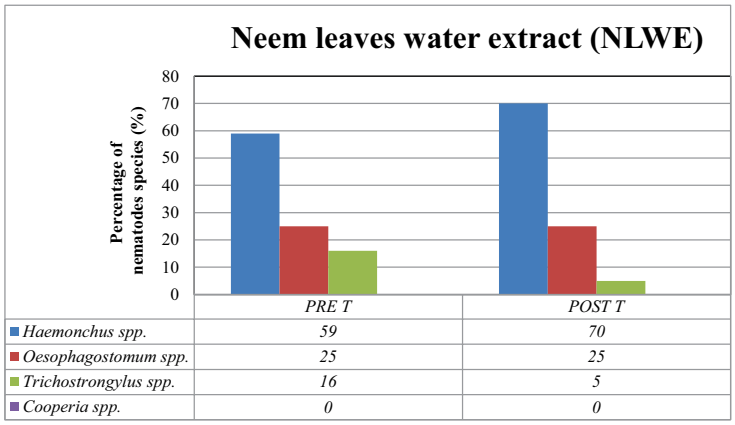


Figure 1. Mean of Egg per Gram (EPG) against week for all control and experimental groups



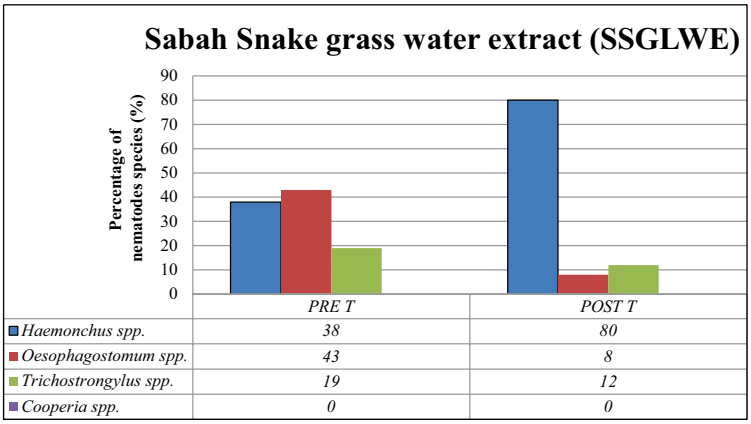
	PRE T	POST T
Haemonchus spp.	46	84
Oesophagostomum spp.	18	6
Trichostrongylus spp.	36	8
Cooperia spp.	0	2

Figure 2a. Percentage of L_3 species in control group of goats for pre- and post-treatments.



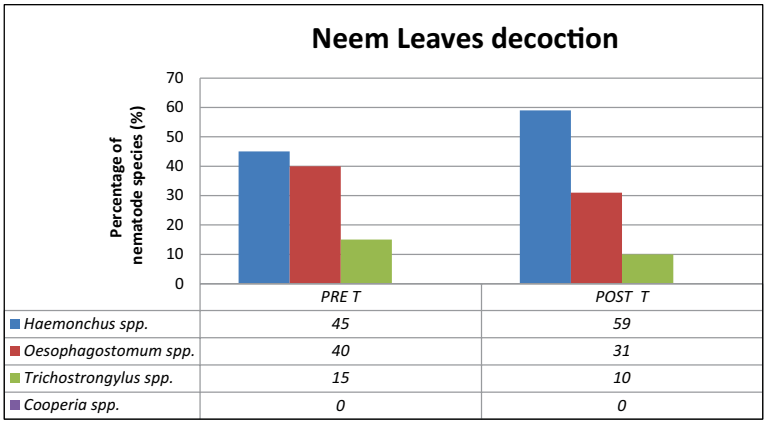
	PRE T	POST T
Haemonchus spp.	59	70
Oesophagostomum spp.	25	25
Trichostrongylus spp.	16	5
Cooperia spp.	0	0

Figure 2b. Percentage of L_3 species in experimental goats treated with Neem (*A. indica*) leaves water extract for pre- and post-treatments



	PRE T	POST T
Haemonchus spp.	38	80
Oesophagostomum spp.	43	8
Trichostrongylus spp.	19	12
Cooperia spp.	0	0

Figure 2c. Percentage of L_3 species in experimental goats treated with Sabah Snake grass (*C. nutans*) leaves water extract for pre- and post-treatments



	PRE T	POST T
Haemonchus spp.	45	59
Oesophagostomum spp.	40	31
Trichostrongylus spp.	15	10
Cooperia spp.	0	0

Figure 2d. Percentage of L_3 species in experimental goats treated with Neem (*A.indica*) leaves decoction for pre- and post- treatments.

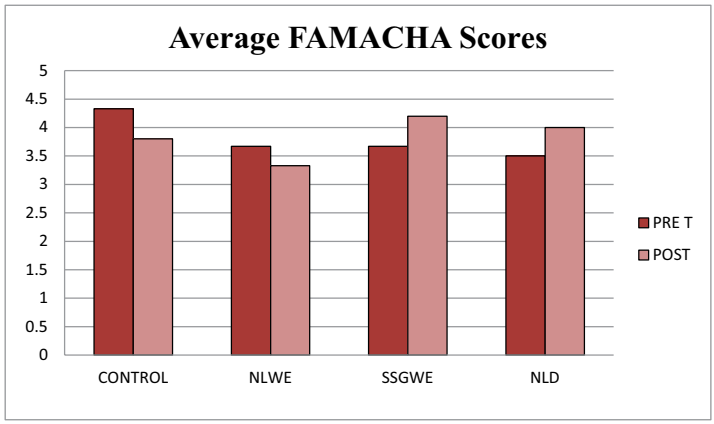


Figure 3. Average FAMACHA Scores of the experimental goats in the control and treatment groups

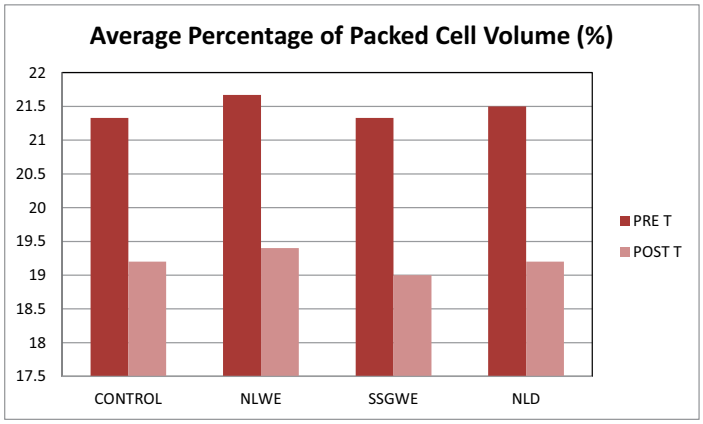


Figure 4. Average Percentage of Packed Cell Volume (% PCV) of experimental goats in the control and treatment groups

second week, mean epg of Sabah Snake grass water extract treated group was 1800. Then the epg count rose drastically and reached 3460 epg which is almost twice the average reading of epg of the second week. However, the reading dropped moderately to 2960 mean epg at the fourth week recording the highest epg among other groups at the end of the study. Control group showed a high epg at the odd week while recording a sudden fluctuation at even weeks recording the lowest mean epg reading of 760 compare to other groups at the final week. Average epg reading of the group treated with Neem leaves water extract decreased gradually for the first three weeks from 1466 (1st week) to 733 (3rd week) and showed a sudden increase at the fourth week (1080 epg). Neem leaves decoction treated group showed a sharp decline at week 3, with a reduction of five times lower epg reading than the second week (2483) only to rose back at week 4 (1060 mean epg).

Figure 2 shows the percentage of L_3 species in all groups of experimental goats for pre- and post-treatment and four species of nematodes were found during the study period which are *Haemonchus* sp., *Oesophagostomum* sp., *Trichostrongylus* sp. and *Cooperia* sp. Generally, *Haemonchus* spp. were found to be the most abundant species in both pre- and post- treatment whereas *Cooperia* spp. was only found in the post-treatment of control group. Percentage of *Haemonchus* sp. did not show any sign of reduction in all four experimental groups while the other two species; *Oesophagostomum* sp. and *Trichostrongylus* sp. declined significantly. Figure 3 shows only the control and NLWE treated group showed improvement by recording FAMACHA score of 3.8 and 3.33 respectively. Herbal treatment of SSGLWE and NLD showed slight deterioration by recording higher degree score at the end of the study. Figure 4 showed that the percentage of Packed Cell Volume (% PCV) declined significantly for all the groups.

Individual faecal egg count (FEC) were arithmetically transformed using ln to ln FEC + 1. Based on Anova One-Way test, the significant value was 0.946 ($P > 0.05$). There were no significant difference between the faecal egg count (FEC) for all group tested. Besides that, Post Hoc Multiple Comparison data analysis showed the significance between the control group and treatments group were almost 1.00.

DISCUSSION

Overall, this study had shown that the Neem leaves water extract, Neem leaves decoction and Sabah Snake Grass leaves water extract did not significantly reduce the faecal egg count in the experimental goats. Free pasture grazing and cut and carry practice greatly affects the results of the study conducted at the private farm which manages the farm semi-intensively. This farm allows the goats to graze at the available pasture area (communal grazing) which may be highly contaminated with third stage infective larvae for about two

to four hours daily. Thus, under traditional free grazing approach, the goats are exposed to continuous infection and re-infection and as grazing area becomes limited they are forced to graze closer to the heavily contaminated pasture (Waller, 2004).

Migration of infective larvae from the faeces to the herbage is necessary and parasites generally adapt in a manner that promotes infection success. High relative humidity and temperature promotes the migration of L_3 of *H.contortus* to all grass height strata as it was found to be the most dominant species in faecal culture of both pre- and post-treatment. For instance, *H. contortus* exhibit the mixed strategy which is a combination of cruising and ambushing strategy where it increases the probability of being ingested by host by exiting the faecal and positioning itself at the tip of vegetation (Fenton *et al.*, 2004). The experimental goats were randomly picked, tagged and allocated into groups with an assumption that the faecal egg counts for all the goats prior to treatment were similar. This could have been overcomed by treating all the experimental animals with anthelmintic before beginning the experiment. The variation in faecal egg count reading would have been significant if the study was initiated with same average epg for all four groups. On top of that, some experimental goats could not be identified according to their group as their ear tag came off due to their aggressive interaction with other goats. A collar tag together with ear tag would have been a great help for identification purpose. The technical error while delivering the extract could have reduced the amount of herbal extract the experimental goats consumed. Additionally, the goats showed high preference towards Sabah Snake Grass leaves water extract due the presence of maltose (70%) in it. The goats also spitted a large portion of the extract during feeding and this maybe because of fear, bitterness in the Neem extract and human force exerted on it. This affects the amount of dosage each goat consumed for each treatment.

A well-planned parasite control must be practiced to reduce and restrict build-up of parasite contamination on pasture. Besides zero grazing, alternate grazing or integrated grazing has been practised for sometime by allowing mixed species grazing. A practical approach would be to allow the cows to graze the same grass pasture before goats. However, this higly depends on decreased susceptibility of the different host species for parasitic nematodes (Stromberg et al., 1999). On top of that, mixed grazing of nematode resistant breed with susceptible breed will reduce the exposure of pasture contamination to susceptible goats (Burke, 2006).

Manipulation of host nutrition is vital to enhance the small ruminant's immune response against the worms and also to resist any adverse effects of gastrointestinal nematode infection (Hoste *et al.*, 2005). According to a study conducted at University of Brazil on sheeps, protein

supplementation in their diet boosted the development of resistance towards gastrointestinal nematodes (Louvandini *et al.*, 2006). It is highly recommended that, supplementary diet is given with plant extracts with anti-parasitic properties such as Neem leaves.

Based on this study, all three herbal treatments exhibited low efficacy against gastrointestinal parasites as the faecal egg count shows the treated group of experimental goats had no significant difference to the control group ($P > 0.05$). This finding contradicts the discovery of the other study which evidently showed that Neem leaves decoction is more effective than Neem leaves water extract in controlling worm burden in local goats (Chandrawathani *et al.*, 2013). Generally, the experiment treated the experimental goats with 20 ml of Neem leaves decoction weekly for a period of 10 weeks, differing from this trial where 50 ml of Neem leaves decoction were given only twice for a study period of 4 weeks. These finding could be due to short period of the study and the frequency of treatment given.

On top of that, experimental goats treated with *Clinacanthus nutans* (Sabah Snake Grass) extract recorded highest average faecal egg count (2,960) at the end of the study period. It has too be mentioned again that the herbal extract given to the goats were a mixture of *C. nutans* (30%) and maltose (70%). Generally, all organisms requires constant supply of glucose in blood to maintain the catabolic process of using glucose to obtain energy in the form of Adenosine triphosphate (ATP). Eosinophills are leukocytes that are responsible to combat parasitic infections and regulate the allergic response in mammals. These cells of myeloid lineage acquire energy entirely via glycolysis (Kominsky *et al.*, 2010). Thus, high amount of glucose is supposed to increase the immune system in the small ruminants and result in reduced faecal egg count.

However, the outcome disapproved the above statement. The large amount of disaccharide in the Sabah Snake Grass Leaves water extract (SSGLWE) resulted in highest average faecal egg count in the 3rd and 4th week of the study (Figure 1). Excessive maltose given may have created a conducive environment and provide energy for the population burst of microbes in the intestinal guts. Colonization of microbes in the gastrointestinal tract is capable of triggering inflammation in the intestinal lining. This further exposes the inflammed lining to the worms and reduces the protective coat against gastrointestinal nematodes. Adult worms inhabiting the intestine can now easily obtain blood and nutrients from the host and direct the energy to boost reproduction. Hence, efficacy of SSGLWE should be determined by using pure extract of SSGLWE in future.

A study reported a strong correlation between faecal egg count, FAMACHA score and PCV (Kaplan *et al.*, 2004). Similar findings was seen in this study, an increase in FEC recorded a decrease in PCV and an increase in the eye score which can be caused by

the high percentage of *Haemonchus contortus (*80%) (Figure 2c) found in the group treated with SSGLWE at the post-treatment. Severity of haemonchosis can be better determined by conducting a faecal occult blood test as initial phase of *Haemonchus contortus* infection has little impact of blood loss (Colditz *et al*., 2008). Hence, both common veterinary practice; FAMACHA and PCV reading had proved that the herbal therapy failed to control the worm burden in the experimental goats.

Based on the results obtained, it can be assumed that the herbal dosage may have been insufficient and the extraction process of plants must be done meticulously to prevent any harm towards the active ingredients. There were no previous studies on *Clinacanthus nutans* regarding anthelmintic properties. Hence, further research has to be done to prove its capability in controlling helminth problem and determine its toxicity level. A longer study of period is necessary to study the behaviour of gastrointestinal parasites in various conditions and effectiveness of the ethnoveterinary approach. Thus, further research has to be done to find out an effective non-chemical strategy for nematode parasite control globally.

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